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Efficacy and mechanism of action of long-term treatment with antibody NI-203.26C11 targeting aggregated IAPP in type 2 diabetes

Inaugural-Dissertation

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Table of contents

1. Zusammenfassung

Typ-2-Diabetes ist gekennzeichnet durch Hyperglykämie, Insulinresistenz, reduzierte Insulinsekretion und β-Zellverlust, welcher mit einer Insel-Amyloid-Polypeptid- (IAPP) Aggregation in den Pankreasinseln einhergeht. Der Prozess der IAPP-Aggregation führt zur Bildung von IAPP-Oligomeren, die für β-Zellen zytotoxisch sind. Es gibt bislang keinen therapeutischen Ansatz, der auf die IAPP-Aggregation abzielt. Aus diesem Grund wurde der Antikörper NI-203.26C11 spezifisch gegen toxische humane IAPP-Oligomere entwickelt. In der Langzeitstudie wurde die Wirksamkeit einer wöchentlichen Behandlung mit NI-203.26C11 (0.3, 1, 3, 10 mg/kg) im Vergleich zu zum inerten Antikörper LALA-PG NI-203.26C11 (10mg/kg) untersucht. Überraschenderweise hatten die transgenen Ratten im Alter von 11 Monaten noch keinen deutlich manifesten Diabetes entwickelt. Trotzdem zeigte sich tendenziell eine Verbesserung der Stoffwechsellage bei den mit NI-203.26C11 (1 bis 10mg/kg) behandelten Tieren. Die Therapie mit LALA-PG rNI.26C11 führte zu einem ähnlichen Ausmaß an Verlust der β-Zellfunktion wie das Vehikel, was bedeutet, dass die Makrophagenaktivierung ein wichtiger Wirkmechanismus für die Entfernung von zytotoxischen hIAPP-Oligomeren ist. Eine Methode zur Sichtbarmachung des sog. Target Engagement wurde unter Verwendung von unfixierten, gefrorenen Pankreasschnitten transgener Ratten erfolgreich entwickelt. Diese Methode ist ein wertvolles Werkzeug für die Visualisierung und Quantifizierung des NI-203.26C11-vermittelten Abbaus von IAPP-Aggregaten. Trotz den noch ausstehenden Ergebnissen zeigen unsere Untersuchungen, dass die Immuntherapie mit NI-203.26C11 in verschiedenen Tiermodellen umfassend validiert werden konnte und ein großes therapeutisches Potenzial zu besitzen scheint.

Schlüsselwörter: Typ-2-Diabetes, HIP Ratten, Antikörpertherapie

2. Summary

Type 2 diabetes is characterized by hyperglycemia, insulin resistance, reduced insulin secretion, and β-cell loss paralleled by islet amyloid polypeptide (IAPP) aggregation in pancreatic islets. The process of IAPP aggregation leads to the formation of IAPP oligomers, which are cytotoxic for β-cells. Up to date, there is no therapeutic approach targeting IAPP aggregation. For this reason, the antibody NI-203.26C11 targeting toxic human IAPP oligomers has been developed. In the long-term study, the efficacy of weekly treatment with NI-203.26C11 (0.3, 1, 3, 10 mg/kg) was assessed in comparison to the inert antibody LALA-PG NI-203.26C11 (10mg/kg). Surprisingly, at 11 months of age, the diabetic phenotype of transgenic rats had not yet progressed sufficiently to observe conclusive differences between treatment groups; however, the NI-203.26C11 (1 to 10mg/kg) treated rats seemed to show improved β-cell function. Therapy with LALA-PG rNI.26C11 resulted to similar loss of β-cell function as vehicle, implying that macrophage activation is a key mechanism of action for the clearance of cytotoxic hIAPP oligomers. A method of in vivo target engagement was successfully developed using unfixed frozen pancreas sections of transgenic rats. This will be a valuable tool for the visualization and quantification of NI-203.26C11-mediated clearance of IAPP aggregates. Despite some pending results, we believe that immunotherapy with NI-203.26C11 has been extensively validated in different animal models and holds great therapeutic potential.

Keywords: type 2 diabetes, HIP rats, antibody therapy

3. Introduction

3.1. Type 2 diabetes

Type 2 diabetes (T2D) is a growing global health concern. An estimated worldwide prevalence of 8.8% of adults (20 to 79 years of age) are suffering from diabetes, which corresponds to a total of 424.9 million affected people. 90% of these cases are due to T2D, which is the most common form of diabetes. More importantly, a further increase is expected in the coming decades (International Diabetes Federation 2017). T2D is linked to both genetic and environmental risk factors (Tuomi et al. 2014). According to a recent review paper (Bellou et al. 2018), associated risk factors include dietary factors such as an increased consumption of processed meat, sugar-sweetened beverages and decrease in whole grain consumption. There was also a positive correlation to obesity (which may be due to the overconsumption of the food items mentioned above) and physical inactivity as well as medical conditions such as the metabolic syndrome or gestational diabetes. Genetic and epigenetic factors also contribute to the manifestation of the pathological characteristics of T2D. Namely, these pathological characteristics of T2D are insulin resistance, defective insulin secretion, hyperglycemia, loss of β-cell mass and function, as well as micro- and macrovascular complications (Stumvoll et al. 2005; Despa et al. 2012) (Figure 1).

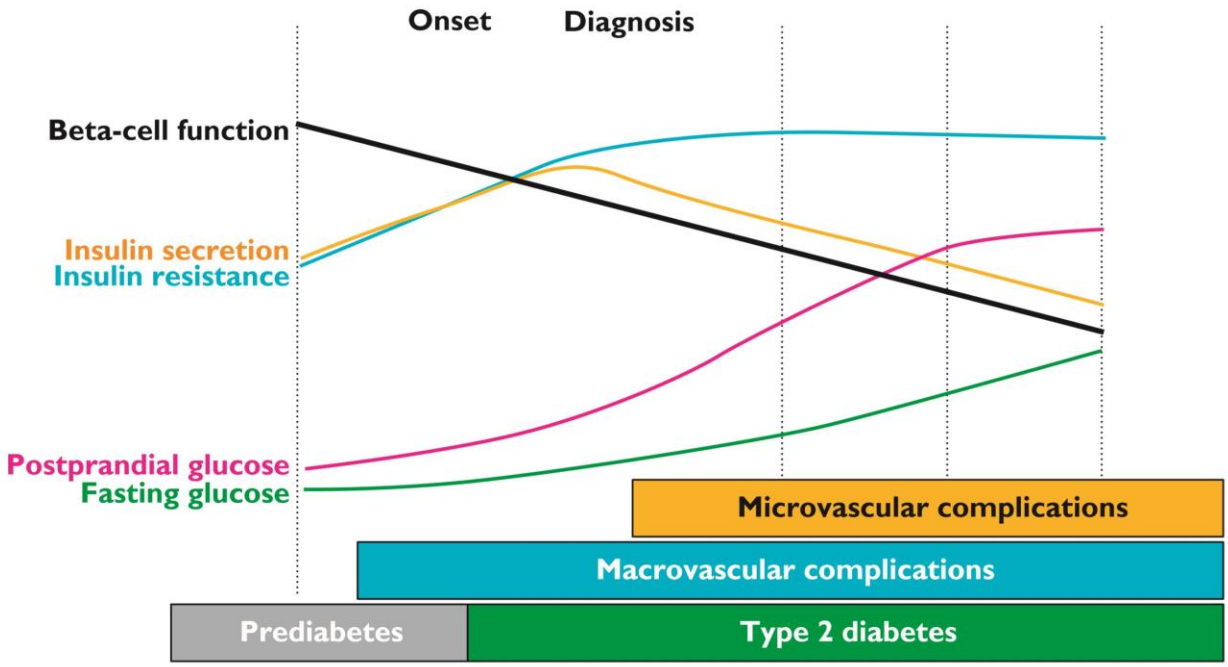


Figure 1: Overview of the development of pathological changes and clinical complications during the progression of T2D (Piya et al. 2010).

Another pathological characteristic is the formation of islet amyloid polypeptide (IAPP) - aggregates in the islets of Langerhans (Stumvoll et al. 2005). Although IAPP aggregation is also seen to a lesser extent in healthy individuals (BELL 1959; Ludwig and Heitner 1967; Westermark 1972), it is a typical morphological feature of T2D and can be observed in most of the people suffering from T2D (Westermark et al. 2011) (Figure 2).

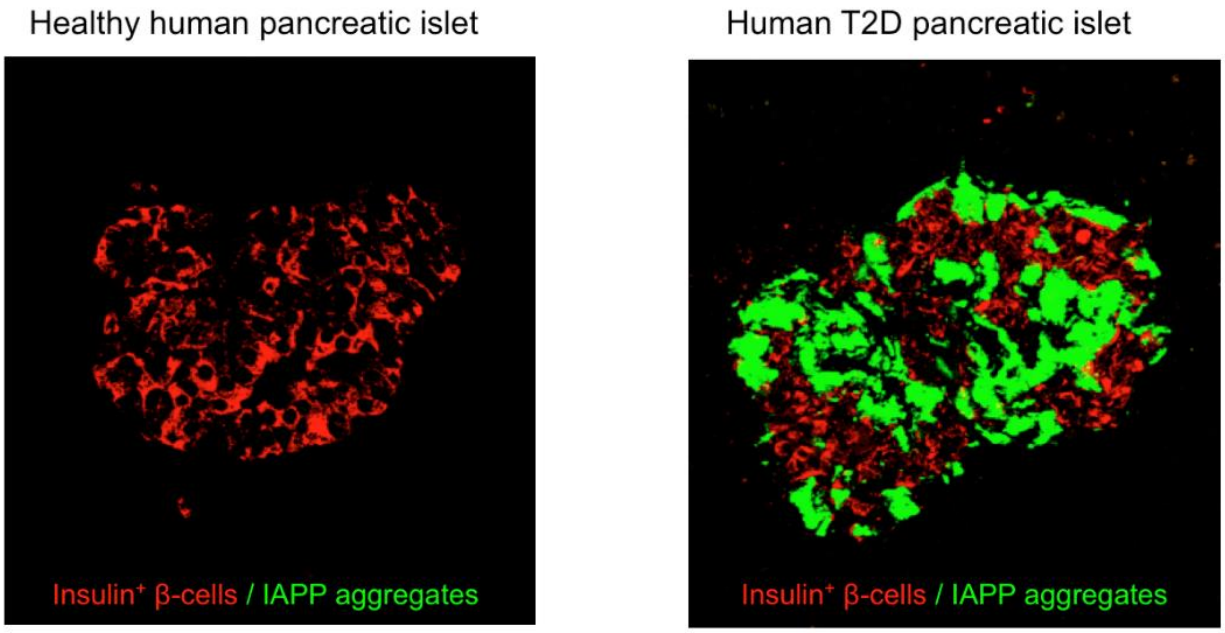


Figure 2: Fluorescent β-cell immunostaining for insulin and IAPP aggregates in pancreatic islets of a health human and a human suffering from T2D in comparison. (Neurimmune AG)

The pathological process of IAPP aggregation results in increased β-cell toxicity (Lorenzo et al. 1994; Jurgens et al. 2011; Haataja et al. 2008; Abedini et al. 2016; Kahn et al. 1999; Jaikaran and Clark 2001; Clark and Nilsson 2004; Hull et al. 2004; Höppener and Lips 2006; Kapurniotu 2001), which contributes to the pathogenesis of T2D. Despite extensive research concerning IAPP aggregation, many open questions remain. For example, the site of initiation, the triggering factors for amyloid formation and the exact mechanisms of cytotoxicity are still unclear.

3.2. IAPP

IAPP is also known as amylin. It is a 37-aa residue long peptide, which is co-secreted with insulin by pancreatic β-cells.

Physiological role of IAPP

Even though the physiological role of IAPP is still not fully understood, the known functions of the monomeric circulating peptide are diverse and involved in several important functions in the organism. High-affinity IAPP receptors are formed when receptor activity-modifying proteins (RAMPs) dimerize with the calcitonin core receptor (Muff et al. 1999; Tilakaratne et al. 2000). IAPP has an inhibitory effect on eating (Arnelo et al. 1996; Barth et al. 2003; Abedini et al. 2018; Lutz 2006; Lutz et al. 1994) and on gastric emptying (Reidelberger et al. 2001). Further, IAPP has been reported to inhibit glycogen synthesis and insulin-stimulated glucose uptake (Cooper et al. 1988) and to inhibit glucose transport (Zierath et al. 1992). The contradictory results regarding the impact of IAPP on insulin secretion indicate a possible dual effect. Further studies also indicate a paracrine or autocrine function of IAPP on pancreatic α-cells to reduce glucagon secretion. Overall, it is widely accepted that IAPP plays a fine-tuning role in metabolism and glucose homeostasis (Westermark et al. 2011).

Introduction

IAPP sequence

The phylogenetically well-preserved IAPP sequence indicates that these physiological functions are of importance (Westermark et al. 2011), yet there are minor but relevant variations between species. For example, human IAPP (hIAPP) and rodent IAPP (rIAPP) differ only by 6 of 37 amino-acid residues (Figure 3). And yet, a high aggregation propensity has been observed in hIAPP in vitro (Kayed et al. 1999; Goldsbury et al. 2000; Padrick and Miranker 2001; Yanagi et al. 2011), while rIAPP has a much smaller propensity towards the formation of fibrils. Variation between species is most common in the 20-29 region of IAPP, 5 out of the 6 differences in amino-acid residues between hIAPP and rIAPP are found there. Proline residues in this region are also known as β-sheet breakers. They occur in rIAPP, but not in hIAPP (Westermark et al. 1990). The lack of these proline residues is strongly associated with amyloid formation. In this respect, it is interesting to note that feline IAPP is similar to hIAPP, and felines also develop diabetes mellitus similar to T2D (Betsholtz et al. 1989; Betsholtz et al. 1990).

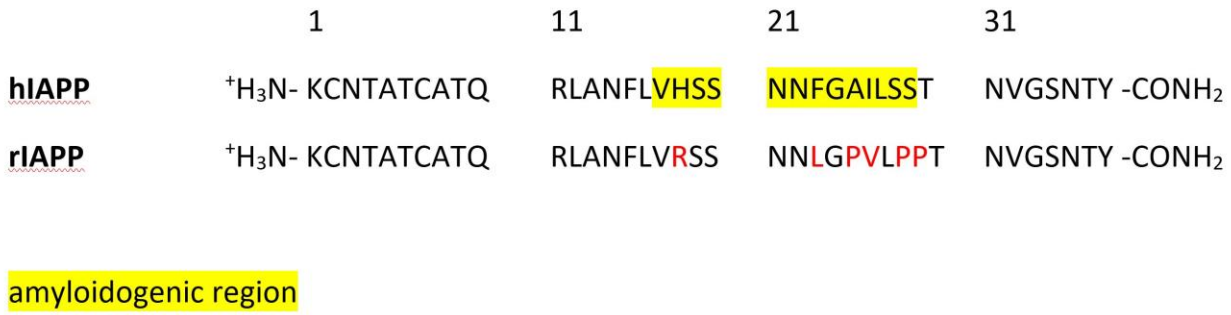


Figure 3: A comparison of the primary sequences of human IAPP (hIAPP) and rodent IAPP (rIAPP), with the six varying amino-acid residues (red) that are found in the amyloidogenic region (yellow).

Amyloid diseases

Amyloid is defined as highly ordered protein or peptide aggregation into cross-β-sheet structures (Sunde and Blake 1997; Greenwald and Riek 2010) in combination with deposited material such as proteoglycans and glycoprotein-serum-amyloid-P-component in vivo (Westermark et al. 2007). T2D belongs to the group of amyloid diseases together with Alzheimer's disease, Parkinson's disease, Huntington's disease and prion disease (Chiti and Dobson 2006, 2017). All of these diseases are caused by the aggregation of specific polypeptides, which are considered to be intrinsically disordered proteins (IDP) with no fixed 3D structure (Uversky et al. 2008). The failure to maintain a functional conformation can result in misfolding, loss of physiological function, aggregation and gain of toxic function (Uversky et al. 2009; Uversky 2010). Therefore, T2D has been proposed to belong to the protein misfolding diseases (Mukherjee et al. 2015).

Process of hIAPP aggregation

In pancreatic islets, hIAPP aggregation into amyloid fibrils occurs through an unknown mechanism (Abedini and Schmidt 2013; Cao et al. 2013; Westermark et al. 2011). New evidence suggests that it might be induced by a prion-like mechanism (Mukherjee et al. 2017). The aggregation of hIAPP can be divided into three stages (Figure 4a) (Abedini et al. 2016). In the lag phase (LP), prefibrillar oligomers are formed and aggregate further into amyloid fibrils during the growth phase (GP). In the saturation phase (SP), there is a balance between soluble hIAPP and amyloid fibrils.

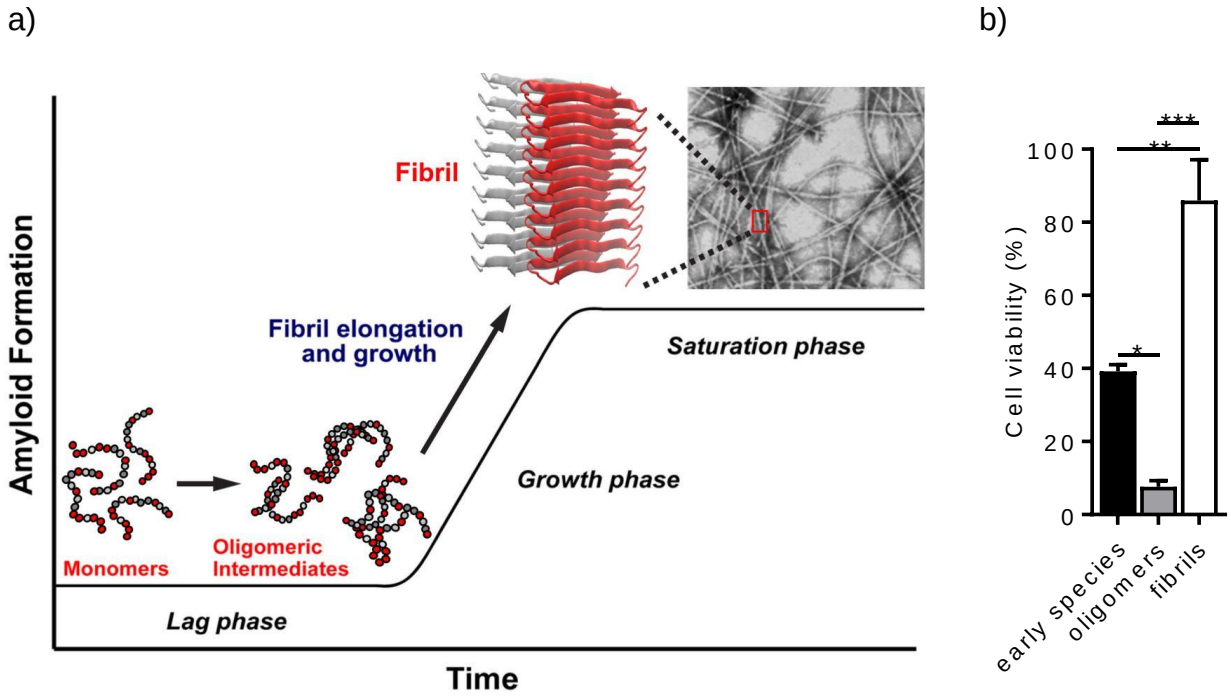


Figure 4a) The 3 phases of amyloid formation: the lag phase, growth phase and saturation phase. The formation of cytotoxic oligomeric intermediates mainly occurs in the lag phase (Abedini et al. 2016) b) Viability of INS-1 β-cells in percentage after incubation with different IAPP species (early species, oligomers, mature fibrils) for 16 hours (Neurimmune AG).

Toxic hIAPP oligomers

The toxic oligomer hypothesis is supported by data showing that β-cell death is caused by prefibrillar toxic oligomers (Janson et al. 1999; Anguiano et al. 2002; Porat et al. 2003; Ritzel et al. 2007; Haataja et al. 2008; Abedini et al. 2016) rather than mature amyloid fibrils (Figure 4b). These toxic IAPP species are transient and form during the lag phase (LP) of amyloid formation. They induce oxidative stress, islet inflammation and β-cell apoptosis (Abedini et al. 2016). Interestingly, not all IAPP oligomers were found to be toxic. For example, the non-amyloidogenic but non-toxic rIAPP also forms oligomers in vitro (Abedini et al. 2016; Young et al. 2014). The same was observed in point-mutated IAPP variants (Abedini et al. 2007; Meng et al. 2010; Wang and Raleigh 2014). This suggests that amino-acid sequence-dependent conformational properties determine cytotoxicity, rather than oligomer formation or size alone (Abedini et al. 2016).

Mechanisms of cytotoxicity

The exact mechanisms of cytotoxicity are still unknown (Westermark et al. 2011; Akter et al. 2016; Abedini and Schmidt 2013). Various studies suggest that β-cell toxicity can be caused by both intra- and extracellular hIAPP oligomers. Possible intracellular mechanisms of cytotoxicity include ER stress (Huang et al. 2007; Casas et al. 2007), mitochondrial membrane damage (Hernández et al. 2018) and defects in autophagy (Kim et al. 2014; Shigihara et al. 2014; Rivera et al. 2011). Extracellular mechanisms encompass various receptor-mediated mechanisms of cytotoxicity linked to oxidative stress (Zraika et al. 2009) and apoptosis (Abedini et al. 2018; Gurlo et al. 2016; Park et al. 2012), activation of the caspase cascade (Zhang et al. 2003) and direct membrane permeabilization and disruption (Gorbenko and Kinnunen 2006; Jayasinghe and Langen 2007; Brender et al. 2012; Gao and Winter 2015; Caillon et al. 2016; Engel 2009). Overall, multiple mechanisms of action may overlap, with possibly many more yet to be described (Abedini and Schmidt 2013). Despite all data indicating the implication of hIAPP in the T2D pathogenesis, there is no treatment targeting IAPP aggregation or protecting β-cells from IAPP-induced toxicity up to now (Ashcroft and Rorsman 2012; Halban et al. 2014)

3.3. Therapeutic approaches

Current therapies mainly aim at lowering blood glucose, but there is no specific treatment available that can halt or reverse the progression of T2D by protecting β-cells (Ashcroft and Rorsman 2012; Halban et al. 2014). Therefore, the development of therapies preventing progressive β-cell loss is of utmost importance. This could be achieved by reducing inflammation or by activation of the unfolded protein response and autophagy that are impaired in T2D (Rojas et al. 2018). Another possible approach to preserve β-cells is to prevent cytotoxicity caused by the process of IAPP aggregation. Since this need has been recognized, numerous therapeutic approaches are being examined toward this aim. Extensive research is currently underway in this field, although conclusive clinical studies are still pending.

Examples of therapeutic approaches

There are many novel approaches concerning the development of compounds aiming at the inhibition hIAPP self-aggregation and neutralization of toxic oligomers. However, many of these studies have not surpassed in vitro experimentation.

One approach is to develop compounds that inhibit hIAPP self-aggregation and neutralize the formation of toxic oligomers. This can be achieved through metal complexes, such as Co(III) (Lee et al. 2012), Cu(II) (Jeong et al. 2010), Al(III) (Xu et al. 2016), Schiff base oxovanadium (Xu et al. 2018) or titanocene complexes (Du et al. 2017).
Chaperones, for example O4 (Zou et al. 2017), rutin (Aitken et al. 2017), serum amyloid A component (Gao et al. 2015), clusterin (Beeg et al. 2016), rationally designed Hsp70 (Bongiovanni et al. 2018), polyphenols and their derivates such as EGCG (Franko et al. 2018; Ehrnhoefer et al. 2008; Xu et al. 2016) or curcumin (Pithadia et al. 2016; Sparks et al. 2012; Daval et al. 2010), as well as calcium-binding nuclebindin-1 (Bonito-Oliva et al. 2017), stabilize the structure of non-aggregated IAPP and thereby prevent aggregation. Short peptides such as macrocyclic peptides (Spanopoulou et al. 2018), hexa-peptides (Scrocchi et al. 2002), D-ANFLVH (Wijesekara et al. 2015) and D-amino-acid (Wang et al. 2014) inhibit amyloidogenesis by mimicking IAPP interaction surface while possessing minimal elements of self- and cross-recognition. To name a few further approaches, the use of nanoparticles (Guo et al. 2013; Yousaf et al. 2017), macromolecules (Seeliger et al. 2013), PEG-PE micelle (Fang et al. 2018),

dendrimers (Gurzov et al. 2016), siRNA (Marzban et al. 2016) and potentially even rIAPP (Cao et al. 2010) to prevent IAPP aggregation have been investigated. Interestingly, it has been proposed that certain amyloid inhibitors paradoxically prolong cytotoxicity by preventing further aggregation, therefore sustaining oligomer toxicity (Abedini et al. 2016). Other novel approaches are not only aimed at targeting aggregation but also at downstream mechanisms of hIAPP-induced cytotoxicity, such as RAGE (Abedini et al. 2018).

Immunotherapy

Immunotherapy is another approach. An important aspect of immunotherapy is the ability of antibodies to both bind specifically to an antigen and to recruit immune effector functions (Wang et al. 2018). Due to their therapeutic potential for many disease complexes, over 70 antibodies underwent late-stage clinical trials within the last year (Strohl 2018). An increasing number of antibodies were approved for marketing within the last 4 years, reaching a new maximum of ten approvals in 2017 (Kaplon and Reichert 2018), with a tendency to rise even further.

First studies examining immunotherapy targeting IAPP aggregates have been conducted. In transgenic hIAPP mice, a vaccination-based approach was found to be overall inconclusive (Lin et al. 2007; Krishnamurthy et al. 2016; Zhang et al. 2018). Also, specific antibodies against cytotoxic IAPP species have been identified in diabetic patients (Bram et al. 2014) and intravenous immunoglobin administration with polyclonal serum IgG was shown to have a protective effect on β-cells (Zhang et al. 2018). Despite these mixed results, the potential of immunotherapy against the toxic effects of hIAPP oligomers is commonly recognized.

3.4. Parallels to Alzheimer research

AD in general

Alzheimer's Disease (AD) and T2D have many shared characteristics. AD is also a widely spread disease, as it is currently affecting 40 million people worldwide (Burns and Iliffe 2009; Prince et al. 2013; Prince et al. 2016), with a rising tendency. As in T2D, there is a long preclinical phase preceding the symptoms of AD (Villemagne et al. 2013). The hallmarks of AD are the misfolding and pathological aggregation of the peptide amyloid β (Aβ) in the brain and the aggregation of hyperphosphorylated protein tau intracellularly (Hardy and Selkoe 2002; Jack et al. 2010; Querfurth and LaFerla 2010). It has been suggested that toxic oligomers of protein misfolding diseases share many common features (Bolognesi et al. 2010; Bucciantini et al. 2002; Chen et al. 2013; Chimon et al. 2007; Glabe 2008; Kim et al. 2009; Laganowsky et al. 2012; Mannini et al. 2014), with Aβ possessing sharing a high similarity to hIAPP (Abedini et al. 2016). This similarity may even lead to cross-seeding behavior between the two amyloids (Andreetto 2010, Nicolls 2004, Zhang 2015). Furthermore, there is literature proposing other pathogenetic links between AD and T2D (Yang and Song 2013; Ninomiya 2014; Oskarsson et al. 2015).

AD and T2D

Comparable to the situation in T2D, AD therapies only consist of symptomatic and transiently active treatments (Schilling et al. 2018). Hence, the interest in the successful development of passive immunotherapy against Aβ arose. One of these antibodies targeting Aβ aggregates is aducanumab, which has been shown to reduce amyloid load and preserve cognitive function repeatedly when chronically administered in AD patients. Aducanumab is a human-derived IgG1 antibody identified by Neurimmune using its proprietary RTM technology platform, similarly to our antibody NI-203.26C11 targeting hIAPP aggregates for the treatment of T2D. The administration of aducanumab was associated with an increased recruitment of microglia, indicating induction of aggregated Aβ phagocytosis in preclinical models of AD. Additional mechanisms such as the neutralization of toxic oligomers are also being considered (Sevigny et al. 2016). Due to the pathological similarities between Aβ and hIAPP aggregates, it seems plausible that aducanumab and NI-203.26C11 antibody may share common mechanisms of action and disease-modifying properties.

3.5. Previous studies using hIAPP directed antibodies

The potential benefits of using antibodies against IAPP aggregates have been recognized in recent years and led to extensive research efforts. Even though antibody-based immunotherapy against IAPP aggregates has yet to be developed and validated, preliminary studies testing the antibody NI-203.26C11 have shown promising results. Upon extensive *in vitro* selection and validation, NI-203.26C11 targeting toxic hIAPP oligomers has been expressed recombinantly for *in vivo* testing in a set of preclinical studies in different animal models of T2D. Among them, five studies have been conducted in hemizygous transgenic rats and mice (HIP rats and mice) expressing amyloidogenic hIAPP under the control of the rat II insulin promoter (Table 1).

For studies in HIP rats, a chimeric version of the antibody NI-203.26C11 composed of rat IgG2b constant regions was administered intraperitoneally in weekly intervals, at doses ranging from 1 to 10 mg/kg and blood glucose levels and plasma insulin levels during oral glucose tolerance tests (OGTTs) have been measured at defined time points. Rat study 1 showed improved glucose tolerance and insulin response in HIP rats treated with NI-203.26C11 (3mg/kg) compared to vehicle. In the second study, efficacy of rNI-203.26C11 (3 mg/kg) was evaluated for a longer time and compared to an IgG control (3 mg/kg). The results of study 1 confirmed rNI-203.26C11 efficacy, with reduced glycemia and β-cell loss. The aim of the third study was to determine the most effective dose of rNI-203.26C11 antibody. For this, three different doses (1, 3 and 10 mg/kg) were applied to hemizygous HIP rats. Although the significant effect of the antibodies on slowing down the progression of T2D was reconfirmed, the 1 and 10 mg/kg doses were equally effective, with no clear dose-dependent effects observed. It was shown that rNI-203.26C11 protects pancreatic β-cells from hIAPP-induced toxicity (Figure 5). After weekly i.p. injections with rNI-203.26C11 (10 mg/kg) in transgenic HIP rats for 40 weeks, an increase in the insulin-positive area as well as β-cell mass was observed. This protection resulted in the preservation of β-cell function, which was reflected by both improved insulin response and decreased blood glucose levels during an oral glucose tolerance test (OGTT;

Figure 6).

Model	*Study*	*Treatment*			*Results of NI-203.26C11 treatment*			
		NI-203.26C11 (weekly i.p.)	*Start*	*Duration*	*Improved glucose tolerance*	*Improved insulin response**	*Reduced glycemia*	*Reduced β-cell loss*
HIP rat [#]	**1**	• 3 mg/kg (n=9) • vehicle (n=10)	12 weeks of age	18 weeks	+	+	NA	NA
	2	• 3 mg/kg (n=11) • IgG (n=12) • vehicle (n=9)	12 weeks of age	30 weeks	+	+	+	+
	3	• 1 mg/kg (n=19) • 3 mg/kg (n=17) • 10 mg/kg (n=18) • vehicle (n=18)	12 weeks of age	40 weeks	+	+	+	+
	4	• 3 mg/kg (n=14) • metformin (n=14) • 3 mg/kg+metformin (n=13) • 3 mg/kg backup AB (n=14) • vehicle (n=14)	12 weeks of age	33 weeks	+	+	NA	NA
HIP mice	**5**	• 10 mg/kg (n=22) • vehicle (n=25)	4 weeks of age	14 weeks	+	+	+	+
Human islet-engrafted mice with HFD-induced diabetes	**6A**	• 10 mg/kg (n=6) • IgG (n=9)	2 weeks post-transplant	12 weeks	+	+	+	NA
	6B	• 10 mg/kg (n=3) • IgG (n=3)	8 weeks post-transplant	6 weeks	+	+	+	NA
Human islet-engrafted mice with STZ -induced diabetes	**7**	• 10 mg/kg (n=16) • IgG (n=14)	at transplant	7-9 weeks	ND	ND	+	+

Table 1: Overview of previous preclinical studies examining the effect of the antibody NI-203.26C11, with the animal model, study design, time and results. [#] Rats obtained from Charles River following embryo transfer and breeding; * Reflects β-cell function; NA = not applicable; ND = not determined (Neurimmune AG)

a) b)

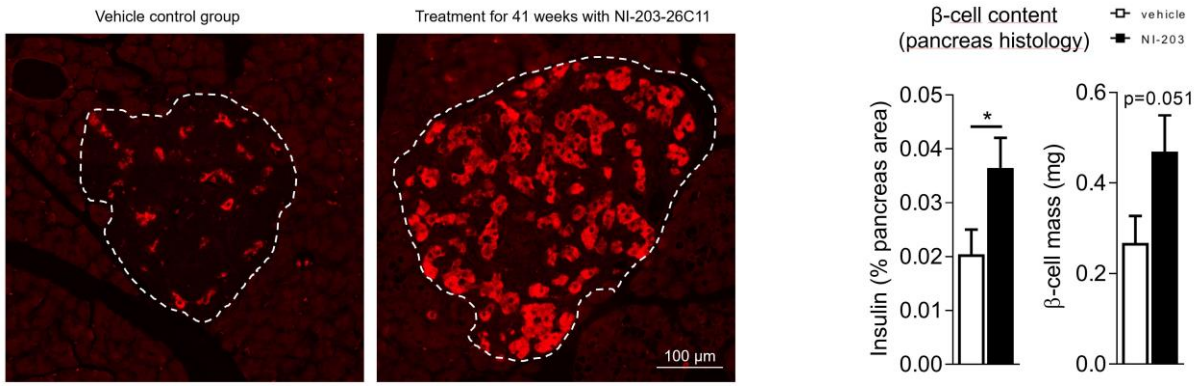


Figure 5: a) Pancreatic islet of PBS- and rNI-20326C11-treated HIP rats using insulin immunostaining to visualize β-cells (1° guinea pig anti-insulin antibody and a 2° TRITC-labeled anti-guinea pig antibody); b) Percentage of insulin-positive pancreas area and β-cell mass in PBS- and rNI-20326C11-treated HIP rats (Neurimmune AG).

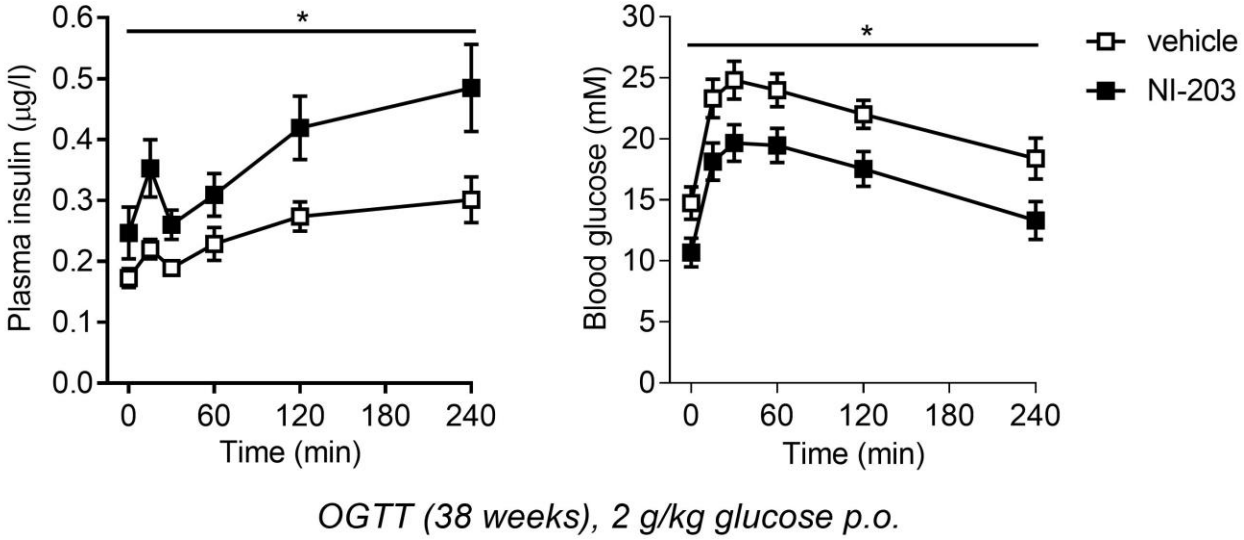


Figure 6: Plasma insulin and blood glucose levels in an OGTT of the HIP rats study 3: significantly improved insulin response and glucose tolerance were observed at all timepoints of the OGTT (oral glucose tolerance test) in rNI-20326C11-treated HIP rats compared to PBS-treated rats after 38 weeks of treatment.

rNI-203.26C11 was also found to recruit macrophages in HIP rat islets. This was shown by pancreas histology with immunostaining of CD68-+ macrophages in pancreatic islets (Figure 7a), which illustrates an increase in macrophage infiltration. This increase in CD68+ islet area was quantified and found to be significant in islets from rNI-203.26C11-treated HIP rats compared to vehicle-treated rats (Figure 7b).

a) b)

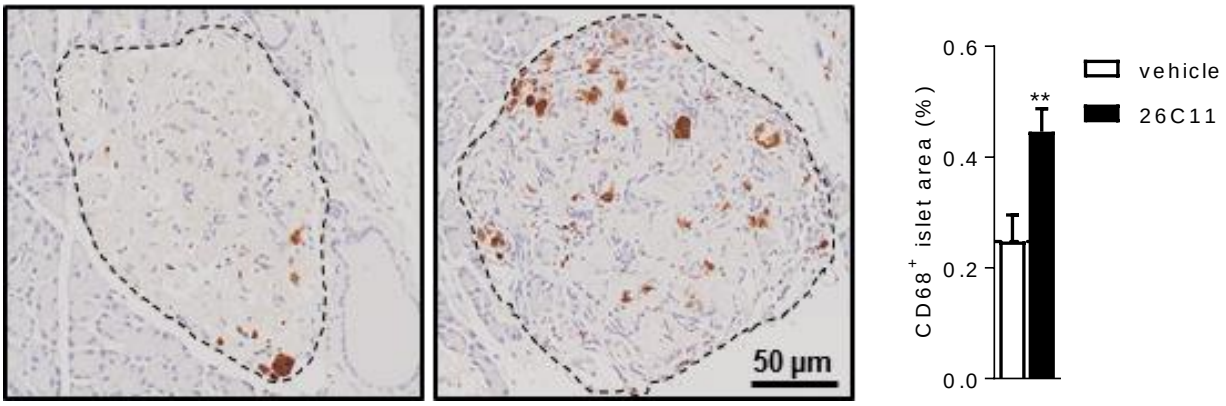


Figure 7: a) Pancreas histology of rNI-20326C11-treated HIP rats compared to vehicle-treated rats upon macrophage immunostaining (using bright field microscopy with 1° rabbit anti-CD68 antibody and 2°anti-rabbit antibody labelled with HRP (horse radish peroxidase); b) Quantification of CD68+ islet area in vehicle-treated HIP rats and rNI-20326C11-treated HIP rats (Neurimmune AG).

It has also been observed that NI-203.26C11 stimulates the uptake of hIAPP aggregates by human macrophages in vitro, suggesting antibody-mediated clearance of hIAPP aggregates by rat macrophages in vivo. This will be discussed in more detail in the next chapter (Inert LALA-PG rNI-203.26C11). This ability to both bind an antigen and simultaneously recruit immune effector functions is an important aspect of antibody therapy (Wang et al. 2018). Following this, rat study 4 compared the lead antibody NI-203.26C11 to the first-line T2D medication metformin and to a slightly different back-up antibody (NI-203.11B12) selectively targeting hIAPP aggregates. NI-203.26C11 was equally effective as metformin in reducing glucose intolerance and led to increased insulin release, suggesting improved β-cell function. NI-203.26C11 and NI-203.11B12 antibodies were equally effective, confirming the pathological role of hIAPP aggregates. Together, these results from preclinical studies in HIP rats led to the conclusion that chronic treatment with NI-203.26C11 antibody protects β-cells. Additional studies performed in hIAPP transgenic mice and in human islet-engrafted diabetic mice showed consistent results to the rat studies. In conclusion, pre-clinical results obtained from different diabetic rodent models indicate that long-term administration of the antibody NI-203.26C11 may preserve β-cell function, resulting in improved glucose tolerance and insulin response. Results also demonstrate a disease-modifying effect of NI-203.26C11 that slows down the progression of T2D.

3.6. Aims

The overall aim of the current study was to investigate the following topics:

1) The long-term effect of four different doses of NI-203.26C11 on β-cell function in glucose intolerant rats;
2) The short-term effect of a single administration of NI-203.26C11 on β-cell function, drug and serum amylin levels in glucose intolerant and hyperglycemic rats;
3) The role of macrophage-mediated clearance of hIAPP aggregates (using an inert and mutated LALA-PG version of NI-203.26C11 antibody);
4) The development of a method for histological visualization and quantification of NI-203.26C11-mediated clearance of hIAPP aggregates.

Based on our previous dose response study (study 3), the minimal therapeutic dose remains unknown. We therefore repeated a dose-response study with weekly i.p. administration of 4 doses of the antibody NI-203.26C11 (0.3, 1, 3, 10 mg/kg). In addition, the short-term effect of a single i.p. administration of antibody NI-203.26C11 (10 mg/kg) on fasting blood glucose, plasma insulin, glucose tolerance and insulin response was examined over a course of 3 weeks. To examine whether NI-203.26C11 mediates clearance of toxic hIAPP aggregates, NI-203.26C11 (10 mg/kg) efficacy was compared to an inert version (LALA-PG NI-203.26C11, 10mg/kg) in both the long- and short-term studies. LALA-PG NI-203.26C11 harbors three mutations in the Fcɣ domain preventing its interaction with Fcɣ-receptors on immune cells and immune responses (Lo et al. 2017). Therefore, the importance of macrophage activation and clearance of hIAPP aggregates was evaluated. The last aim was the development of a method for visualization and quantification of hIAPP clearance *in vivo*. Target engagement will be used as a mean to verify pharmacologically that NI-203.26C11 engages with pathogenic IAPP, enabling a correlation between target engagement and drug efficacy (Simon et al. 2013).

4. Material and methods

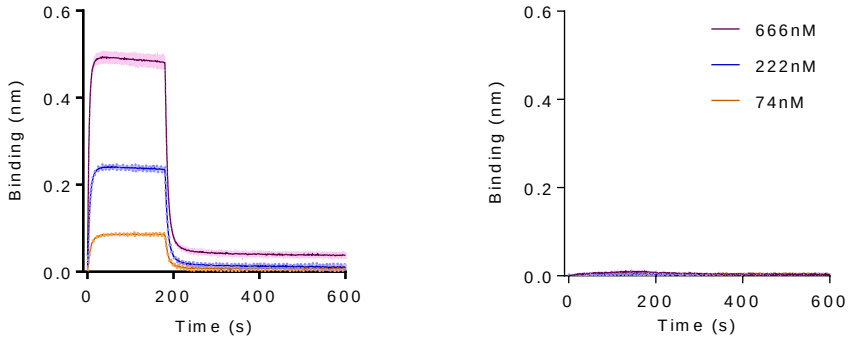
4.1. NI-203.26C11 antibody

The production of the antibody NI-203.26C11 was already described in detail previously (Hugentobler 2017). Neurimmune AG (Schlieren, Switzerland) developed the antibody which was used in this study through the following steps: human-derived monoclonal IgG1 antibodies produced by reactive memory β-cells were isolated from healthy elderly people and first screened by ELISA to identify antibodies selectively targeting pathological hIAPP aggregates. Selected antibodies were cloned using PCR and recombinantly produced in CHO (Chinese hamster ovary) cells. Selectivity of these recombinant antibodies toward pathological IAPP was further tested by ELISA, bio-layer interferometry and immunostaining on human diabetic and non-diabetic tissues. The lead candidate antibody NI-203.26C11 was chosen based on its high binding affinity (K_D = 1.5 ± 0.1 nM) and specificity to pathological hIAPP aggregates. While NI-203.26C11 preferentially binds to pre-fibrillar hIAPP oligomers and to fibrils in a lesser extent, it does not recognize non-pathological IAPP species. This specificity results from binding a conformational epitope exclusively exposed on hIAPP aggregates. A chimeric rat version of NI-203.26C11 antibody (rNI-203.26C11) was developed to minimize the immune response against the human part of the antibody using rat models. The rat chimeric antibody version is formed by combining the human IgG1 variable regions responsible for binding to the antigen, combined with rat IgG2b constant regions. The antibody NI-203.26C11 has an IgG-like PK profile with a $t_{1/2}$ (half-life) of 8 days in rats and mice. All antibodies were injected at a volume of 2 ml/kg.

4.2. Inert LALA-PG rNI-203.26C11

For this study, an inert variant of the rNI-203.26C11 antibody was produced to examine the mechanism of action more closely. The Fc-region of an antibody engages both humoral and cellular components of the immune system. This can result in complement-dependent cytotoxicity (CDC), antibody-dependent, cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and inflammation through cytokine secretion. These reactions can be prevented by using an inert variant of the antibody (Lo et al. 2017), in which interaction with the Fcγ-receptor is inhibited by point mutations in the Fc-region. For example, substituting the amino acids L234A, L235A (Chappel et al. 1991) and P329A (Idusogie et al. 2001) residues leads to a “LALA-PG” variant of the antibody. This inert variant of the antibody cannot interact with Fcγ-receptors on the surface of immunocompetent cells, thus preventing an Fc-mediated immune response. Therefore, both complement-binding and antibody-dependent, cell-mediated cytotoxicity and activation of phagocytosis by macrophages are prevented, while maintaining the antibody’s characteristic pharmacokinetics and ability to bind to specific IAPP oligomers (Figure 8). This mechanism is translatable to human IgG1 (Schlothauer et al. 2016) and murine IgG2a (Lo et al. 2017) antibodies.

a)



b)

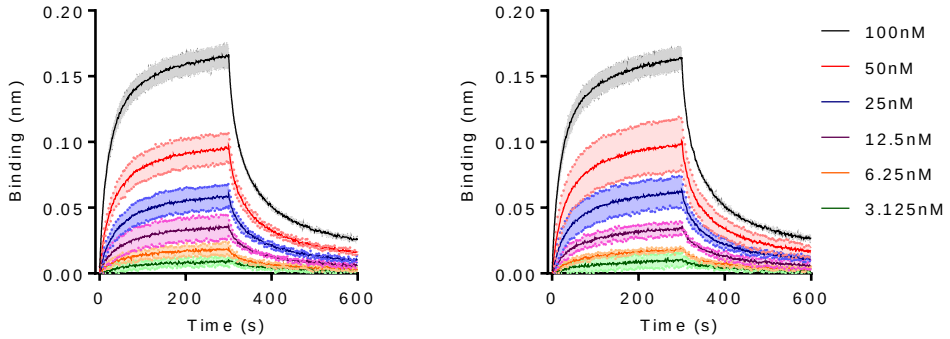


Figure 8: The binding properties of rNI-203.26C11 compared to LALA-PG rNI-203.26C11: a) binding profile of rNI-203.26C11 (left) compared to LALA-PG rNI-203.26C11 (right) on the rat Fcγ-receptor CD64; b) Binding profile of rNI-203.26C11 (left) and LALA-PG rNI-203.26C11 (right) to hIAPP aggregates (Neurimmune AG).

4.3. Animal model

Transgenic rats expressing hIAPP, also known as HIP or RIPHAT rats, were selected as the animal model for this study. The reason for this was the pathological similarities to human T2D in relation to islet amyloid aggregation and the loss of β-cells (approx. 50% at time of onset of diabetes). The spontaneous development and lifelong progression of diabetes in HIP rats correspond to those in humans suffering from T2D (Butler et al. 2004). After a prediabetic phase with impaired first phase insulin secretion and glucose intolerance, HIP rats develop diabetes between an age of 5-10 months, characterized by hyperglycemia and progressive hepatic and extrahepatic insulin resistance as well as hyperglucagonemia (Matveyenko and Butler 2006).

As a control, wild-type rats of the same background were included. The genotype was determined by Charles River. Fasting blood glucose and plasma insulin levels of the transgenic (Tg) rats were compared to those of the wild type (WT) rats before and during the experiment to confirm a diabetic phenotype. Additionally, ThioS staining of amyloid deposits in pancreatic sections was also performed to confirm the genotype at the end of the study. Of the 85 Tg HIP rats ordered at Charles River, 7 were excluded from the study. 15 of the 14 WT rats that had been ordered were included in the study.

4.4. Animals and maintenance

A total of 100 male rats were obtained from Charles River Laboratories (Wilmington, MA, USA) at an age of 8 weeks. Of these, 85 were hemizygous transgenic rats expressing hIAPP (Tg, Crl:CD (SD)) and 15 were wild type rats (WT, Cr:CD (SD)).
Rats were kept under standardized living conditions consisting of a 12:12 hour light-dark cycle (light phase from 02:00 to 14:00) with an ambient temperature of 21 ±1°C degrees. They had ad libitum access to water and chow diet (Extrudate 3436, KLIBA NAFAG, Kaiseraugst, Switzerland) and were group-housed under enriched conditions in standard cages (Type 2000P, 612x435x216mm). The health status of the facility was defined as conventional. The rats were given an acclimatization period of 4 weeks. The initial groups of up to six rats per transport cage were split into groups of 2-3 animals at the age of 13 weeks. At the age of 12 weeks, they were randomized into treatment groups based on their fasting glucose levels of the baseline OGTT. Rats were handled twice per week and subjected to a weekly health check and weighing.

The Veterinary Office of the Canton of Zurich approved of all procedures involving the animals (license nr. 143-2015). The maximal expected degree of severity was 2. End-point criteria were defined as a weight loss > 20% or severe and lasting dehydration. In case of this severe diabetic phenotype or other unexpected suffering (for example self-mutilation, apathy, pain), euthanasia was performed by an overdose of pentobarbiturates (60mg/kg) followed by exsanguination. This occurred in 5 cases, which included a deep reaching open tail wound, dyspnea and three cases of severe weight loss. These animals were excluded from analysis. Two additional animals were excluded from the experiments due to misgenotyping by the commercial provider. No spontaneous deaths occurred up to the time point where this dissertation was written.

4.5. Study design

Two main experiments were performed. After an initial baseline OGTT (12 weeks of age), a dose response (DR) study was conducted for a total time span of 32 weeks to confirm dose-dependent efficacy. In this time span, a total of 7 OGTTs were performed. Additionally, an inert rNI-203.26C11 antibody (LALA-PG) was tested with the aim to shed light on a possible mechanism of action involving macrophage activation and clearance of hIAPP aggregates by phagocytosis. After the DR study, a study on the pharmacokinetic and pharmacodynamic (PK/PD) profile of the antibody rNI-203.26C11 was planned over 3 weeks, including an additional OGTT; however, the latter study could not be performed before the writing of this dissertation due to some delays in the DR study. Nonetheless, the concept of the PK/PD study will be described below. Common factors of these two studies are the animals, which are reused partially, the tested antibodies (rNI-203.26C11 and LALA-PG) as well as other parts of the methodology.
DR study. The aim of the dose response (DR) study was to determine to which extent the effects of the antibody treatment differ between four dosages of rNI-203.26C11 (0.3, 1, 3, 10 mg/kg) compared to PBS and the inert LALA-PG rNI-203.26C11 antibody (10 mg/kg).

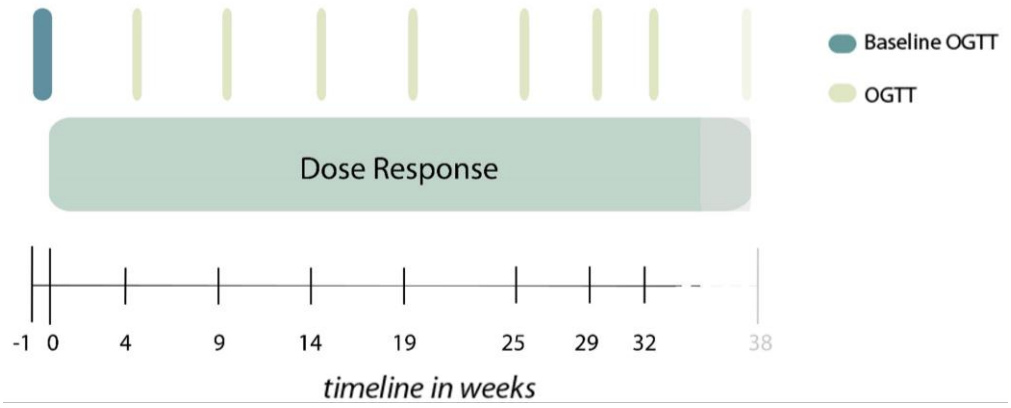


Figure 9: Timeline of the dose response (DR) study including the initial baseline OGTT (dark green) as well as the seven OGTTs (light green) every 3 to 6 weeks. The area in gray represents the future OGTT, as the DR was prolonged beyond the phase described in this thesis.

This study was conducted in 92 rats from an age of 12 weeks over a period of 32 weeks; the study was extended beyond that time point for up to 38 weeks (Figure 9), but here, we will only report results up to the 32 week time point. The 92 rats consisted of 14 WT and 78 Tg rats divided into 7 treatment groups (Table 2). The Tg rats were divided into 6 groups for the testing of varying doses (0.3, 1, 3, 10 mg/kg) of rNI-203.26C11 antibody at and of the LALA-PG (10 mg/kg) compared to PBS vehicle. The WT group was also treated with PBS.

Treatment groups	*Genotype*	*Number of rats*
0.3mg/kg rNI-203.26C11	Tg	10
1mg/kg rNI-203.26C11	Tg	10
3mg/kg rNI-203.26C11	Tg	8
10mg/kg rNI-203.26C11	Tg	8
10mg/kg LALA-PG rNI-203.26C11	Tg	10
PBS	Tg	32
PBS	*WT*	14

Table 2: Numbers and genotypes of rats per treatment groups in the DR study (Tg = transgenic, WT = wild type).

All rats were subjected to weekly antibody or PBS injections (i.p.) from an age of 13 to 37 weeks. A baseline OGTT took place at 12 weeks of age followed by OGTTs performed with an interval of three to six weeks (weeks 4, 9, 14, 19, 25, 29 and 32 after treatment start). Blood samples were collected during the OGTTs to measure fasting blood glucose (FG), fasting plasma insulin (FI) and insulin response (IR) as well as fasting plasma IAPP levels (Table 3).
Blood glucose and plasma insulin levels were measured at the baseline OGTT (-1 week) and during the following OGTTs at weeks 4, 9, 14, 19, 25 and 32 after treatment start. Baseline IAPP were measured at the baseline, 2nd, 4th, 5th and 7th OGTT. In the last OGTT that will not be reported here, where anti-drug response and drug levels will be determined (12 months of age).

	Baseline OGTT	*1st OGTT*	*2nd OGTT*	*3rd OGTT*	*4th OGTT*	*5th OGTT*	*6th OGTT*	*7th OGTT*
Weeks of age	12	17	22	27	32	38	42	45
Weeks of treatment	-1	4	9	14	19	25	29	32
Months	3.5	4.5	5.75	7	8	9	10	10.75
Additional parameters	FG, GR, FI, IR, baseline IAPP	FG, GR, FI, IR	FG, GR, FI, IR, baseline IAPP	FG, GR(Ritzel and Butler 2003, 2003, 2003)	FG, GR, baseline IAPP	FG, GR, FI, IR, baseline IAPP	FG, GR	FG, GR, FI, IR, baseline IAPP

Table 3: Overview of OGTTs, the corresponding age and weeks of treatment as well as the examined parameters. FG: fasting glucose; FI: fasting insulin; IR: insulin response., GR: glucose response.

At the end of the DR study, the PBS-treated WT (n=14) and PBS-treated Tg (n=32) rats will be reused in the PK/PD study. The remaining animals will be sacrificed after the last OGTT (12 months of age approximately) and used for histological assessment of amyloid load in the pancreas.

PK/PD study

This pharmacokinetics and -dynamics (PK/PD) study will focus on the acute effect of a single administration of rNI-203.26C11 (10 mg/kg) or LALA-PG (10 mg/kg) over the duration of 3 weeks (Figure 10) in old rats (12 months of age) with a diabetic phenotype, characterized by glucose intolerance and hyperglycemia.

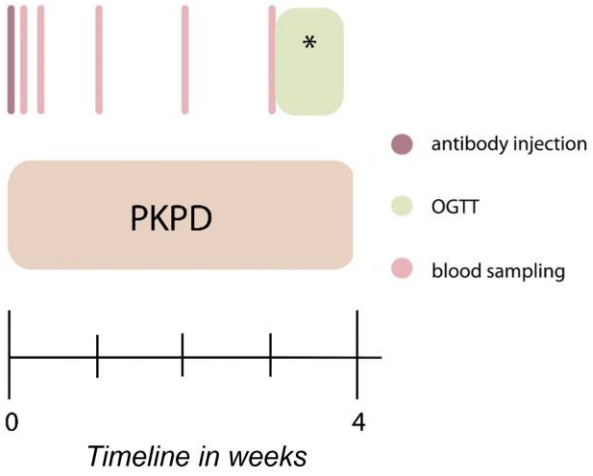


Figure 10: Timeline of the pharmacokinetic and pharmacodynamic (PK/PD) study with a single i.p. administration of rNI-203.26C11, LALA-PG rNI-203.26C11 or PBS, followed by repeated blood sampling (1d, 2d, 3d, 7d, 14d, 21d) and a final OGTT (*).

The aim of this study is to determine the distribution of the rNI-203.26C11 (10 mg/kg) and LALA-PG (10 mg/kg) antibodies in plasma and their acute efficacy on physiological indicators of β-cell function such as blood glucose, plasma insulin and IAPP levels at different time points after a single i.p. injection in transgenic rats. rNI-203.26C11 (10 mg/kg) will be compared to LALA-PG rNI-203.26C11 (10 mg/kg) and PBS vehicle. A total of 32 animals will be included in the study, all of which were previously treated with PBS in the DR study (10 WT, 22 Tg). This

results in 7 to 10 rats per group (Table 4). In all groups, plasma samples will be taken before (0d) as well as at various time points after the single injection (1d, 2d, 3d, 7d, 14d, 21d). The rats will be allocated to the different treatment groups based on fasting blood glucose levels during the 8th OGTT performed at the end of the DR study. The PK/PD study is planned to take place directly after the DR study.

Treatment groups	*Genotype*	*Number of rats (n)*
rNI-203.26C11 (10mg/kg)	Tg	7
LALA-PG rNI-203.26C11 (10mg/kg)	Tg	7
PBS	Tg	8
PBS	WT	10

Table 4: Numbers and genotypes of rats allocated to the different treatment groups for the PK/PD study (Tg = transgenic, WT = wild type).

4.6. Oral glucose tolerance test (OGTT)

The aim of the OGTT was to evaluate glucose intolerance in rats, quantified by the clearance capability of ingested glucose. An initial baseline OGTT was performed in the DR study just before the first administration of antibodies, at an age of 12 weeks. Then, an OGTT was performed 7 times in a time interval of 3 to 6 weeks (ages 17, 22, 27, 32, 38, 42, 45 weeks).

The same protocol was followed as described previously (Hugentobler 2017). After a 12h fasting period (19:00 to 07:00, of which the last 5h were in the light phase), 2 g/kg of glucose (50% glucose, B. Braun, Melsungen, Germany) was administered by oral gavage. Blood was collected from the sublingual vein (20G needle) upon brief anesthesia with isoflurane (3-4%, Attane, Piramal Enterprises Limited, Mumbai, India) to determine blood glucose levels prior to and 15, 30, 60, 120 and 240 minutes after the glucose administration. Blood glucose levels were determined by Contour XT glucometer and glucose stripes (Contour, Bayer, Basel, Switzerland). Additional blood was collected in tubes (350 µl full blood/500 µl EDTA Microtainer K2E tube, Becton Dickinson, Franklin Lakes, USA) for plasma extraction to analyze plasma insulin (ELISA kit Mercodia 10-1250-01) and IAPP levels (MERCK-Millipore EZHA-52K).

Anti-drug antibody and NI-203.26C11 levels will also be measured by ELISA in plasma samples from the last OGTT of the DR study and the PK/PD study. For the determination of anti-drug antibody levels, a Corning 3690 plate was coated with 10 µg/ml h26C11 in PBS O/N followed by incubation of a 1:5000 dilution of rat plasma, detection with rat IgG-specific and HRP-conjugated secondary antibodies (SouthernBiotech) and measurement of HRP activity using standard procedure. For determination of plasma levels of rNI-203.26C11 and LALA-PG rNI-203.26C11, the Corning 3690 plate was coated with 1 µg/ml of a previously validated anti-idiotypic Fab antibody generated by HuCAL technology and selectively recognizing NI-203.26C11 (AbD23344.1, AbD Serotec) in PBS O/N RT. Non-specific binding sites were blocked for 1h at RT with a blocking buffer (2 % BSA, 0.1 % tween-20 in PBS buffer pH 7.4). Plasma samples were diluted 1:30’000 in PBS. Calibration samples were prepared similarly by diluting rNI-203.26C11 antibody in PBS with a concentration range from 1.37 pM to 1 nM. Samples were then incubated for 1h at RT, and rNI-203.26C11 was detected with HRP-conjugated anti-rat IgG2b (gamma chain specific) and anti-rat kappa (kappa chain specific) secondary antibodies (SouthernBiotech) mixed at a 1:5000 dilution in PBS, followed by measurement of HRP activity using standard procedure.

The samples were analyzed using a SUNRISE plate reader (Firmware: 3.32 08/07/08; XFLUOR4(VERSION1) Version: 4.51) except for amylin, which was analyzed using a Thermo VARIOSCAN LUX plate reader.

4.7. Target engagement

A further aim was to visualize NI-203.26C11-mediated clearance of IAPP aggregates. Due to the transient nature of hIAPP oligomers targeted by NI-203.26C11 and their sensitivity to fixation, standard immunostaining approaches were not suitable for the tissue detection of IAPP aggregates using NI-203.26C11. Therefore, an alternative approach relying on in vivo target engagement was developed to visualize and quantify hIAPP aggregates recognized by NI-203.26C11. The development of this method provides a useful tool for future research.

Immunodetection of hIAPP aggregates has been achieved recently upon in vivo target engagement in HIP transgenic mice with a NI-203.26C11-derived fluorescently labeled diabody administered at 30 mg/kg i.p., followed by pancreas harvesting after 2 days and imaging of fluorescently labeled diabody on fixed frozen sections. A diabody is a fusion protein that consists of two dimerized single chain variable light and heavy chain fragments (Holliger et al. 1993). In this case, the diabody consisted of fragments of the variable regions of NI-203.26C11, resulting in similar binding properties compared to the parent antibody towards hIAPP aggregates (data not shown). In general, diabodies were shown to have a very high affinity (Adams et al. 1998). Tissue detection of the NI-203.26C11-derived diabody was combined with counterstaining using a commercial antibody towards physiological monomeric hIAPP. Specificity of the NI-203.26C11-derived diabody toward aggregated hIAPP structures and absence of binding to monomeric hIAPP was shown (Figure 11). In the WT, no target engagement was observed. Unfortunately, for cost and practicality reasons, this fluorescently labeled diabody is not suitable for use in HIP rats, where a large amount would be required due to the higher body weight. Therefore, the aim was to develop a more suitable approach.

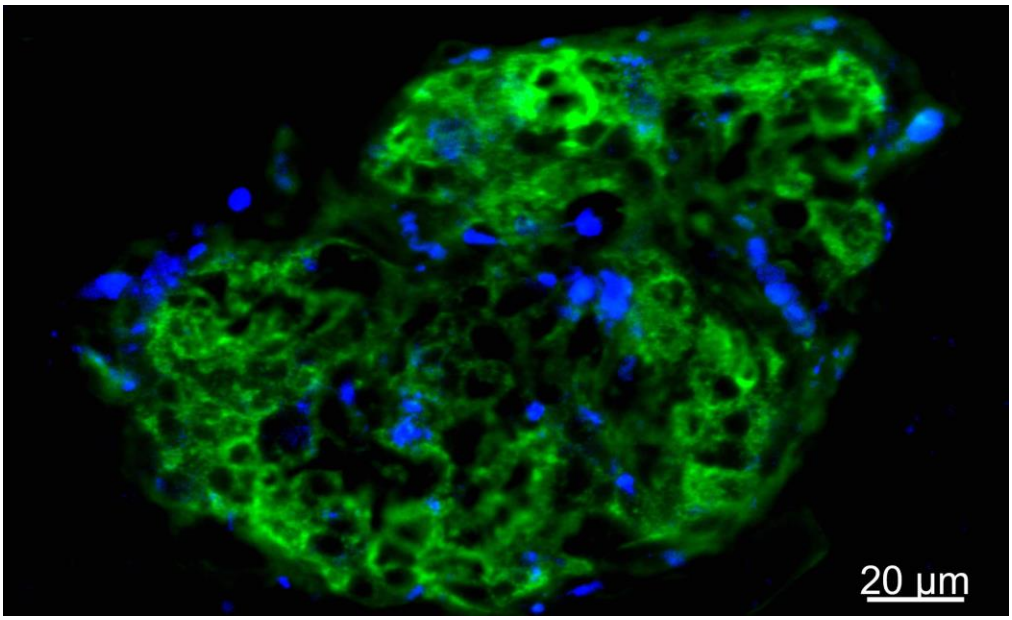


Figure 11: Immunofluorescent staining of a pancreatic islet of a HIP mouse with a commercial anti-hIAPP antibody (green) and a NI-203.26C11-derived fluorescently labeled diabody (blue; Neurimmune AG).

For immunodetection of hIAPP aggregates by in vivo target engagement in HIP rats, human LALA-PG NI-203.26C11 antibody was administered at 30 mg/kg and rats were sacrificed 3 days post-injection. Rats were anesthetized using pentobarbital (60mg/kg i.p.). Then pancreas tissue was removed, immerged in 30% sucrose in PBS for a few hours until submersion, frozen in OCT (Tissue Freezing Medium, LEICA BIOSYSTEMS) and stored at -80°C. Frozen pancreas sections (5 µm) were incubated in blocking buffer (PBS + 5% serum (horse/goat) + 4% BSA) for 1h at RT, followed by incubation with Alexa488-conjugated anti-human secondary antibody (Jackson ImmunoResearch; 1:200) overnight at 4°C. Sections were counterstained with polyclonal guinea pig anti-insulin antibody (1:2; FLEX; Dako) and TRITC-conjugated goat anti-guinea pig secondary antibody (Jackson ImmunoResearch; 1:200). Slides were mounted using Hydromount media (National Diagnostics). Fluorescent slides were imaged using a VS120 slide scanner (Olympus Life Science).

4.8. Quality control

The quality was ensured at various time points by a broad range of measures. The study was blinded by assigning random variables (Q, T, V, W, Z for the DR study) to the treatment groups, while the PBS were defined as such. A lab book was kept, to ensure both reproducibility and traceability.

4.9. Statistics

For statistical analysis and graphical illustration of the data, GraphPad Prism 7 (La Jolla CA, USA) was used. For the analysis of the bodyweight, fasting glucose and insulin, glucose tolerance and insulin response, a two-way ANOVA with a Tukey's multiple comparison test was used. Animals with missing values were excluded. The values of the WT and Tg rats in the baseline OGTT were compared using unpaired T-test assuming Gaussian distribution. The area under curve (AUC) of the groups was computed using a one-way ANOVA with a Tukey's multiple comparison test and the group means, which calculated using the AUC of each individual animal at each timepoint. Significance was defined by a p-value of <0.05 and the data is presented as mean (M) ± standard error of mean (SEM).

5. Results

5.1. Animals

Overall, no generalized polyuria and polydipsia (PU/PD) was observed in the Tg rats up to an age of 45 weeks. Individual animals developed a pronounced diabetic phenotype and a bodyweight loss of >20% at 43 weeks of age and had to be euthanized prematurely, i.e. after 30 weeks of treatment. The respective rats belonged to three different treatment groups (3mg/kg, 10mg/kg, PBS) and were excluded from the statistical analysis. No obvious difference was observed in the phenotype of the different treatment groups including the LALA-PG treated rats. As expected, no diabetic symptoms occurred in the WT rats.

5.2. Body weight gain

During the whole experiment, all rats were weighed weekly over a time span of 37 weeks. The body weight (BW) gain was compared between the treatment groups at all time points. Overall, the groups gained weight continually and only some animals lost weight occasionally or generally in the week following the OGTTs, respectively. There was a significant difference in BW (g) in the Tg PBS compared to the WT PBS rats from as early as 14 weeks of age. At 45 weeks of age, the mean BW (g) of the Tg PBS rats was 13.5% lower compared to that of the WT rats (mean difference of 102.1g).

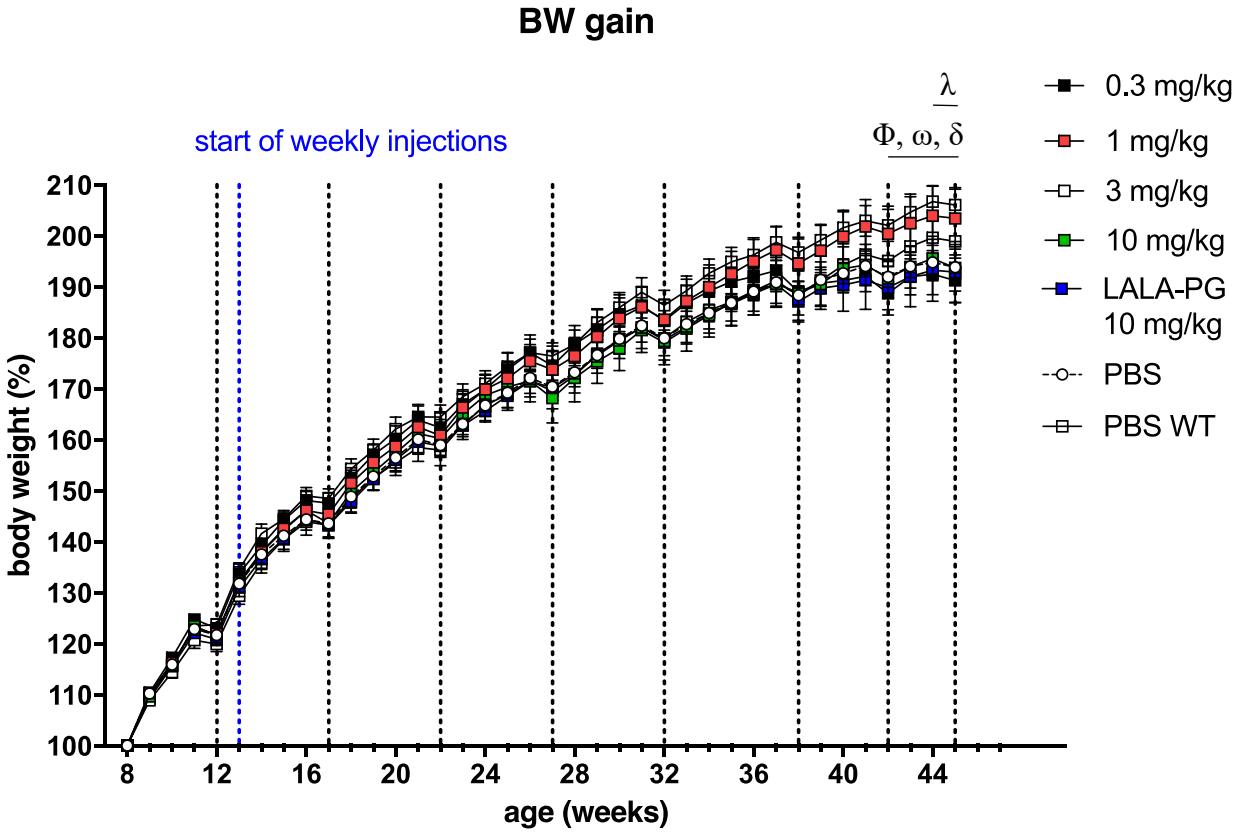


Figure 12: BW gain (%) of the 7 groups in comparison over 37 weeks. The OGTTs are indicated with black grid lines, the start of the weekly antibody injections with a blue grid line. See legend of symbols for significant differences.

Results

Between the different Tg treatment groups and WT group, there was no difference in BW gain (%) up to 42 weeks of age (Figure 12). From week 42 onwards, there was a significant decrease in BW gain in the 0.3mg/kg, LALA-PG 10mg/kg and Tg PBS treated groups compared to the WT PBS. In the Tg PBS group, the decrease in BW gain progressed and became highly significant compared to the WT PBS group from 43 weeks of age on. In the 0.3mg/kg Tg group, the decrease in BW gain became highly significant compared to the WT PBS from 44 weeks of age onwards. In week 45, additionally, the 10mg/kg group showed a significant decrease in BW compared to the WT.

5.3. Dose response study

In total, 8 OGTTs were held in the DR study in rats aged between 12 and 45 weeks. The rats received weekly injections from 13 to 45 weeks of age, for a total of 32 weeks. The following symbols were used to express the significant differences between the groups (Table 5):

	Tg PBS	***Tg 0.3mg/kg***	***Tg 1mg/kg***	***Tg 3mg/kg***	***Tg 10mg/kg***	***Tg LALA-PG 10mg/kg***
Compared to WT PBS (Tg and WT)	Φ	δ	ε	χ	λ	ω
Compared to Tg PBS (only Tg)	-	α	η	μ	π	*
Quantity of symbols indicates grade of significance, for example: ***WT PBS vs. Tg PBS:*** **Φ: p<0.05, ΦΦ: p<0.01, ΦΦΦ: p<0.001, ΦΦΦΦ: p<0.0001**						

Table 5: Legends of symbols used for significant differences in graphs.

Fasting glucose

Fasting glucose (FG) was measured in each OGTT, starting with the baseline OGTT (12 weeks of age), up to the last OGTT (45 weeks of age, i.e. after 32 weeks of treatment). In the baseline OGTT, there was no difference between the WT and Tg rats. From the baseline OGTT to the 3rd OGTT after the start of treatment, no significant differences were observed between the Tg treatment groups and the WT group. From the 4th up the 7th OGTT, the 0.3mg/kg, LALA-PG 10mg/kg and PBS Tg groups showed a highly significantly increase in FG compared to the WT PBS. In addition, the 3mg/kg group had a significantly increased FG in the 4th OGTT and the 1mg/kg group in the 6th OGTT compared to WT PBS. Within the treatment groups, no consistent significant changes were observed. The 10mg/kg group had significantly lower FG levels than the Tg PBS and LALA-PG groups in the 4th OGTT. In the 7th OGTT, the LALA-PG group had significantly higher FG levels than the Tg PBS and the 1mg/kg groups (Figure 13, Table 6).

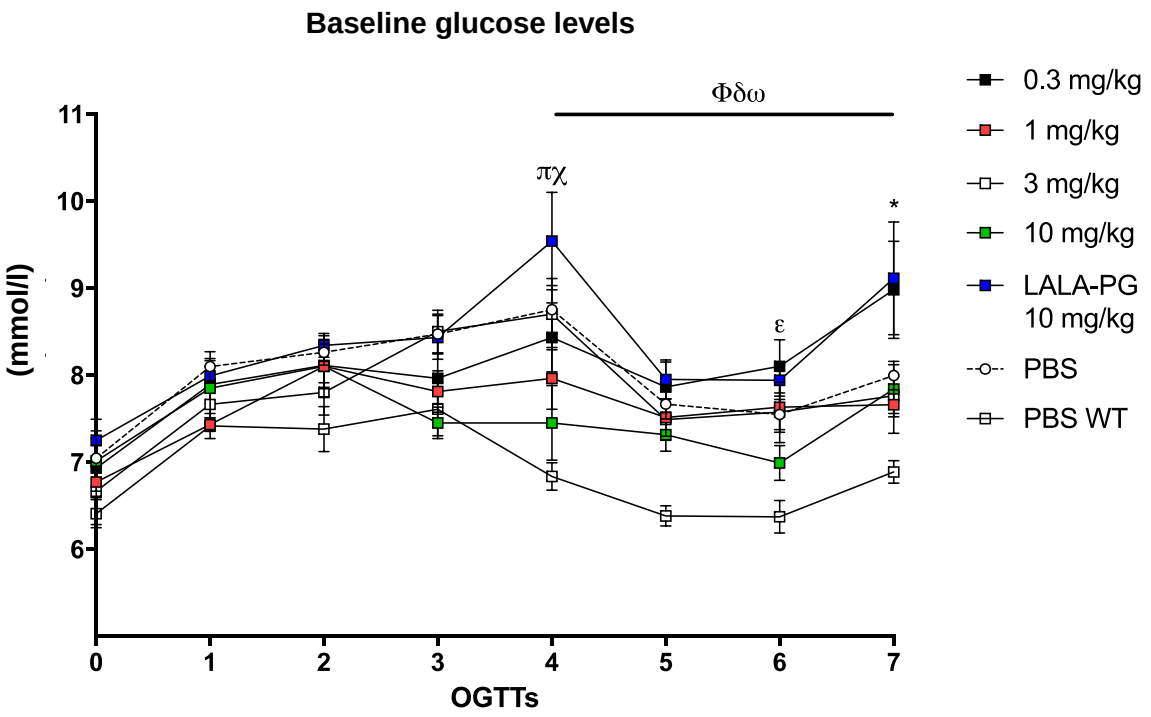


Figure 13: FG (fasting glucose) levels in mmol/l over time measured before each of the eight OGTTs. See legend of symbols for significant differences.

OGTT ***(WOT)***	*WT* *(mmol/l)*		*Tg PBS* *(mmol/l)*		*Tg 0.3mg/kg* *(mmol/l)*		*Tg 1mg/kg* *(mmol/l)*		*Tg 3mg/kg* *(mmol/l)*		*Tg 10mg/kg* *(mmol/l)*		*Tg LALA-PG* *(mmol/l)*		*Significance*
Baseline	6.41	±0.16	7.04	±0.15	6.93	±0.34	6.77	±0.17	6.66	±0.38	7.01	±0.35	7.25	±0.24	-
1st (4)	7.41	±0.15	8.10	±0.17	7.89	±0.21	7.43	±0.08	7.66	±0.29	7.85	±0.34	7.99	±0.17	-
2nd (9)	7.38	±0.26	8.26	±0.15	8.11	±0.20	8.10	±0.26	7.80	±0.26	8.10	±0.35	8.34	±0.14	-
3rd (14)	7.61	±0.31	8.47	±0.23	7.96	±0.29	7.81	±0.24	8.50	±0.25	7.45	±0.18	8.43	±0.25	-
4th (19)	6.84	±0.16	8.75	±0.28	8.43	±0.40	7.96	±0.35	8.70	±0.41	7.45	±0.43	9.54	±0.56	π δδ χχχ φφφφ ωωωω
5th (26)	6.38	±0.12	7.66	±0.17	7.86	±0.29	7.51	±0.22	7.49	±0.24	7.31	±0.19	7.95	±0.23	δδ ωω φφφ
6th (29)	6.37	±0.19	7.55	±0.17	8.10	±0.31	7.63	±0.29	7.58	±0.35	6.99	±0.20	7.94	±0.18	ε ωω φφ δδδ
7th (32)	6.89	±0.13	7.99	±0.16	8.98	±0.56	7.66	±0.33	7.76	±0.24	7.84	±0.28	9.11	±0.65	* φφ δδδδ ωωωω

Table 6: Means and standard error of means of fasting glucose (FG) in mmol/l of all groups in different OGTTs. See legend of symbols for significant differences. WOT= weeks of treatment.

Fasting insulin

The same samples were used to examine fasting insulin (FI) at the baseline (OGTT before start of treatment) as well as the 1st, 2nd, 5th and 7th OGTT. Overall, the WT PBS had higher FI levels than most of the Tg groups in all OGTTs, apart from the baseline OGTT. In the baseline OGTT, no differences could be seen between the FI of the WT and Tg rats. Overall, increased FI levels were observed in the WT rats compared to the Tg 0.3 and 3mg/kg groups as well as the Tg PBS group from the 1st OGTT onward. From the 2nd OGTT on, the LALA-PG group also had a decreased FI level compared to the WT PBS, the same occurred with the 10mg/kg group from the 5th OGTT onwards. The 1mg/kg Tg group only had significantly decreased FI values compared to the WT PBS in the 2nd OGTT. Despite this, the FI levels between the different treatment groups remained comparable except for the last OGTT. There, the 0.3mg/kg treated group had significantly higher FI levels than the Tg PBS group (Figure 14, Table 7).

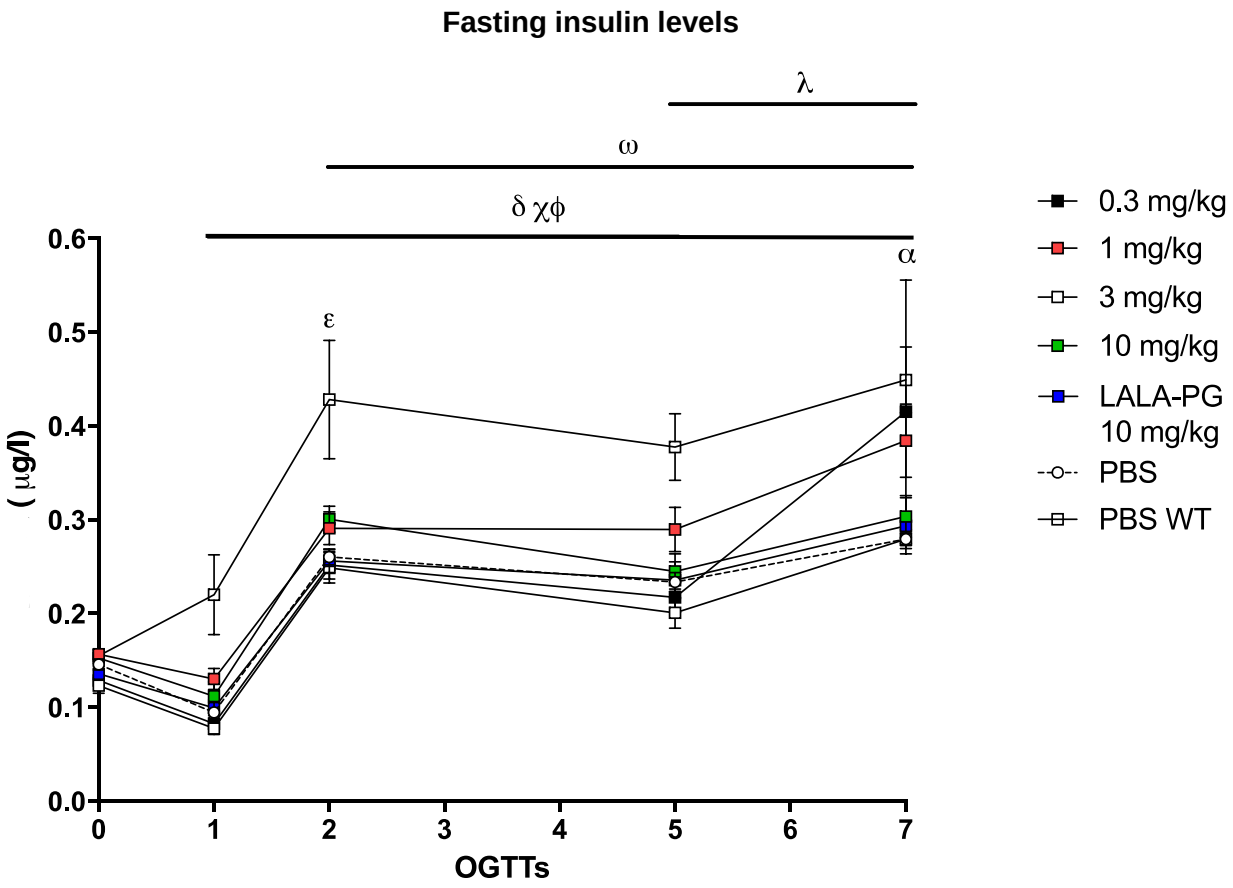


Figure 14: FI (fasting insulin) levels in µg/l over time measured at the baseline, 1st, 2nd, 5th and 7th OGTT (over 32 weeks). See legend of symbols for significant differences.

OGTT (WOT)	*WT (µg/l)*	*Tg PBS (µg/l)*	*Tg 0.3mg/kg (µg/l)*	*Tg 1mg/kg (µg/l)*	*Tg 3mg/kg (µg/l)*	*Tg 10mg/kg (µg/l)*	*Tg LALA-PG (µg/l)*	*Significance*
Baseline	0.16 ±0.01	0.15 ±0.01	0.13 ±0.00	0.16 ±0.01	0.12 ±0.01	0.15 ±0.01	0.14 ±0.01	-
1st (4)	0.22 ±0.04	0.09 ±0.01	0.08 ±0.00	0.13 ±0.01	0.08 ±0.01	0.11 ±0.02	0.10 ±0.01	δ χ ϕϕ
2nd (9)	0.43 ±0.06	0.26 ±0.01	0.25 ±0.01	0.29 ±0.02	0.25 ±0.02	0.30 ±0.01	0.26 ±0.01	ε δδ χχχ ωωω ϕϕϕϕ
5th (29)	0.38 ±0.04	0.23 ±0.01	0.22 ±0.02	0.29 ±0.02	0.20 ±0.02	0.24 ±0.02	0.24 ±0.02	λ ω δδ χχ ϕϕϕ
7th (32)	0.45 ±0.04	0.28 ±0.01	0.42 ±0.14	0.38 ±0.04	0.28 ±0.01	0.30 ±0.02	0.29 ±0.03	λ χ δδ ωω αα ϕϕϕϕ

Table 7: Means and standard error of means of fasting insulin (FI) in µg/l of all groups of rats in different OGTTs. See legend of symbols for significant differences. WOT = weeks of treatment.

Glucose tolerance

The glucose response was examined in all eight OGTTs. From as early on as the baseline OGTT onwards, the Tg rats had an impaired glucose tolerance compared to the WT rats (time points 30, 60, 120 minutes). From the 1st to the 4th OGTT, the glucose levels of 3mg/kg group were significantly lower than those of the Tg PBS group in either the 30- or 60-minute time points, or both. Also, except for the 7th OGTT, a significant decrease in glucose levels was observed in the 3mg/kg compared to the LALA-PG 10mg/kg group in all OGTTs at the 60- and/or 120-minute time points. In the last OGTT, no significant difference in glucose tolerance or in glucose AUC was seen at any time point between the Tg groups. Compared to the WT rats, all Tg groups showed significantly higher glucose levels at the 15-minute time point, except for the 3 and 10mg/kg groups. All Tg groups had significantly higher glucose levels at the 30-, 60- and 120-minute time points. At the 240-minute point, only the 0.3mg/kg and Tg PBS groups still had elevated glucose levels compare to the WT group (Table 8, Table 9Figure 15).

Within the Tg rats, no difference was observed in the baseline glucose levels or AUC during the OGTT done at baseline. The glucose AUC of the 0.3mg/kg, LALA-PG 10mg/kg and Tg PBS groups was increased significantly compared to the WT PBS in all the OGTTs. From the 2nd OGTT on, the 1mg/kg group also had an increased glucose AUC compared to the WT PBS. The AUC glucose of the 10mg/kg group was increased only once compared to the WT PBS, namely in the 3rd OGTT. The AUC glucose of the 3mg/kg group was never increased significantly compared to the WT PBS (Table 9, Figure 16).

Results

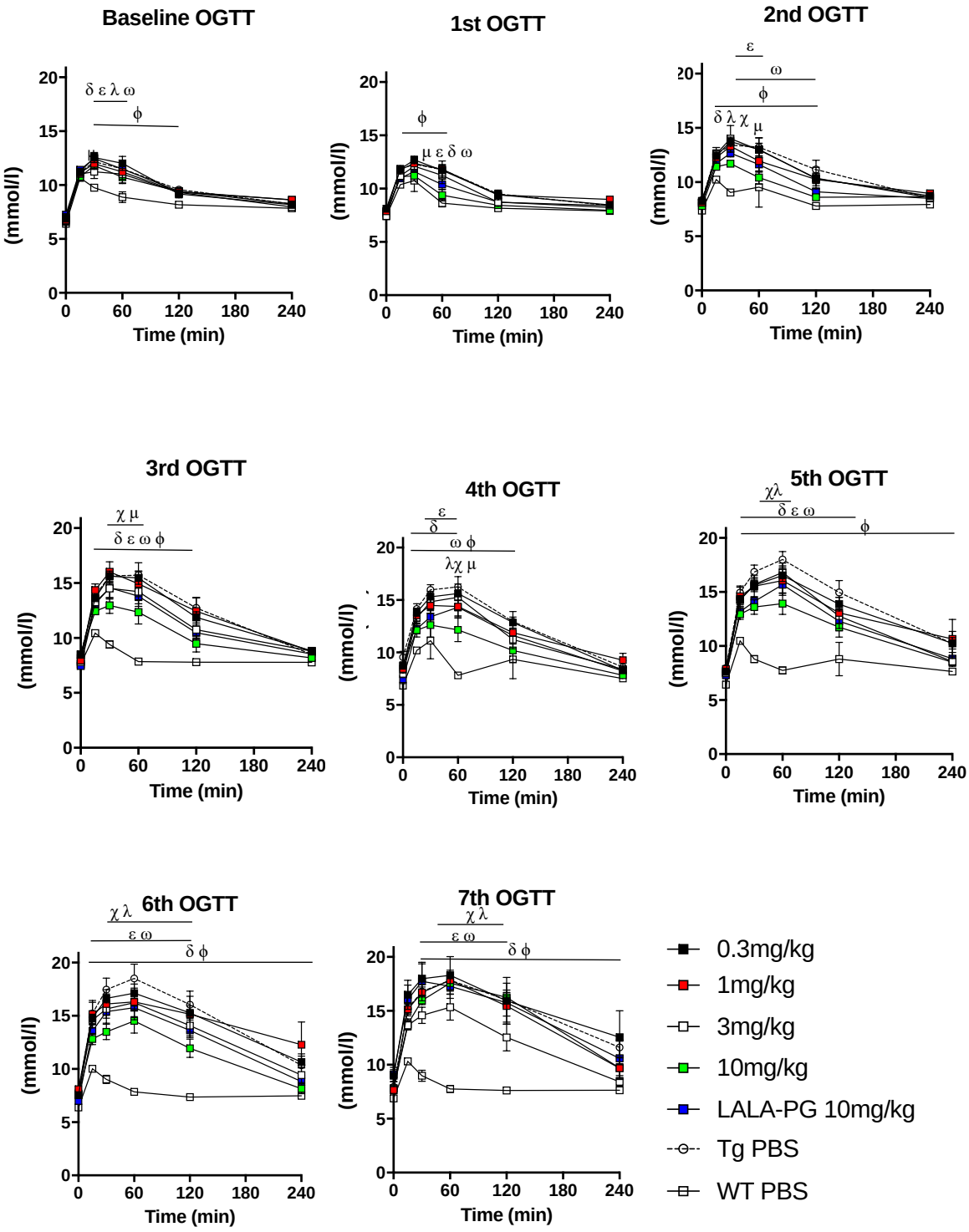


Figure 15: Comparison of glucose curves (mmol/l) at different time points in all OGTTs for each group. See legend of symbols for significant differences.

	(min)	*Tg 0.3mg/kg (mmol/l)*		*Tg 1mg/kg (mmol/l)*		*Tg 3mg/kg (mmol/l)*		*Tg10mg/kg (mmol/l)*		*Tg LALA-PG 10mg/kg (mmol/l)*		*Tg PBS (mmol/l)*		*WT PBS (mmol/l)*		*Significances*
baseline OGTT **(-1 WOT)**	0	6.93	±0.34	6.77	±0.17	6.66	±0.38	7.01	0.35	7.25	±0.24	7.02	±0.15	6.41	±0.16	-
	15	11.24	±0.27	11.14	±0.32	11.04	±0.30	10.78	0.35	11.41	±0.22	11.16	±0.19	10.67	±0.13	-
	30	12.55	±0.48	11.97	±0.54	11.24	±0.63	11.80	0.57	12.46	±0.48	12.18	±0.30	9.76	±0.22	λλ εεε ωωωω δδδδ φφφφ
	60	12.02	±0.66	11.23	±0.68	11.01	±0.88	10.74	0.52	11.48	±0.40	11.51	±0.35	8.89	±0.49	λ χχ μμ εεε ωωωω δδδδ φφφφ
	120	9.18	±0.36	9.36	±0.41	9.33	±0.39	9.41	0.28	9.43	±0.36	9.59	±0.28	8.18	±0.16	ϕ
	240	8.19	±0.32	8.64	±0.24	7.96	±0.25	8.61	0.25	8.26	±0.13	8.25	±0.14	7.85	±0.11	-
1^{st} OGTT **(4 WOT)**	0	7.89	±0.21	7.43	±0.08	7.66	±0.29	7.85	0.34	7.99	±0.17	8.11	±0.16	7.41	±0.15	-
	15	11.77	±0.39	11.19	±0.28	11.13	±0.24	10.99	0.33	11.82	±0.28	11.83	±0.23	10.36	±0.16	ϕ
	30	12.36	±0.54	12.10	±0.52	11.21	±0.48	11.59	0.44	12.30	±0.25	12.69	±0.36	10.75	±1.02	φφ
	60	11.82	±0.78	11.24	±0.77	9.39	±0.55	10.39	0.48	11.78	±0.45	11.72	±0.53	8.64	±0.35	μ εεε δδδδ ωωωω φφφφ
	120	9.41	±0.46	8.73	±0.38	8.44	±0.27	8.75	0.47	9.51	±0.32	9.43	±0.30	8.17	±0.14	-
	240	8.98	±0.27	8.40	±0.15	7.96	±0.18	8.24	0.23	8.41	±0.13	8.49	±0.14	7.90	±0.17	-
2^{nd} OGTT **(9 WOT)**	0	8.11	±0.20	8.10	±0.26	7.80	±0.26	8.10	0.35	8.34	±0.14	8.25	±0.15	7.38	±0.26	-
	15	12.25	±0.52	12.59	±0.60	11.41	±0.22	11.71	0.36	12.58	±0.41	12.43	±0.26	10.23	±0.15	ϕ
	30	13.31	±0.54	14.01	±1.20	11.70	±0.27	12.68	0.35	13.42	±0.66	13.73	±0.43	9.04	±0.24	λλ χχ εεεε δδδδ ωωωω φφφφ
	60	11.94	±1.04	12.97	±1.13	10.43	±0.62	11.64	0.65	13.22	±0.84	13.02	±0.59	9.53	±1.84	μ εεε ωωω φφφφ
	120	10.25	±0.59	10.37	±1.01	8.60	±0.24	9.11	0.36	11.15	±0.87	10.32	±0.46	7.80	±0.13	ωω φφ
	240	8.95	±0.21	8.57	±0.23	8.66	±0.25	8.50	0.31	8.53	±0.19	8.73	±0.18	7.94	±0.11	-
3^{rd} OGTT **(14 WOT)**	0	7.96	±0.29	7.81	±0.24	8.50	±0.25	7.45	0.18	8.43	±0.25	8.48	±0.22	7.61	±0.31	-
	15	14.36	±0.57	13.26	±0.43	12.43	±0.30	13.13	0.61	13.87	±0.45	13.63	±0.29	10.44	±0.21	ε ωω δδδ φφφφ
	30	16.02	±0.92	14.51	±0.87	12.95	±0.72	14.58	1.03	15.64	±0.63	15.64	±0.46	9.41	±0.33	μ χχ δδδδ εεεε λλλλ ωωωω φφφφ
	60	14.94	±1.17	14.22	±1.10	12.34	±1.08	13.80	0.93	15.72	±1.12	15.46	±0.59	7.84	±0.23	μμ χχχχ δδδδ εεεε λλλλ ωωωω φφφφ
	120	12.44	±1.27	10.75	±0.86	9.48	±0.76	10.48	0.47	12.74	±0.91	11.87	±0.51	7.80	±0.13	ε δδδδ ωωωω φφφφ
	240	8.77	±0.32	8.79	±0.22	8.19	±0.20	8.54	0.26	8.49	±0.25	8.84	v0.20	7.78	±0.07	-
4^{th} OGTT **(19 WOT)**	0	8.43	±0.40	7.96	±0.35	8.70	±0.41	7.45	0.43	9.54	±0.56	8.73	±0.27	6.84	±0.16	-
	15	13.53	±0.57	13.29	±0.48	12.11	±0.65	12.25	0.47	14.15	±0.48	13.83	±0.39	10.19	±0.19	δ ωω φφφ
	30	14.49	±0.75	14.84	±0.87	12.60	±1.15	13.39	0.67	15.97	±0.50	15.30	±0.53	11.12	±1.73	ε δ ωωω φφφφ
	60	14.37	±0.99	15.25	±1.28	12.16	±1.13	14.31	0.82	16.25	±0.98	15.61	±0.63	7.81	±0.30	μ χχ δδδδ εεεε λλλλ ωωωω φφφφ
	120	11.89	±1.09	11.22	±0.87	10.20	±1.15	11.63	0.54	12.95	±0.97	12.86	±0.54	9.34	±1.85	ω φφ
	240	9.26	±0.65	8.39	±0.34	7.84	±0.26	8.23	0.34	8.60	±0.63	8.29	±0.23	7.52	±0.21	-

Results

5th OGTT (25 WOT)	0	7.86	±0.29	7.51	±0.22	7.49	±0.24	7.29	0.22	7.95	±0.23	7.63	±0.17	6.42	±0.12	-
	15	14.58	±0.97	14.26	±0.46	12.95	±0.47	13.17	0.48	14.93	±0.51	14.31	±0.37	10.48	±0.17	ε δδ ωω φφφ
	30	15.54	±1.02	15.68	±0.68	13.60	±0.66	14.11	0.46	16.85	±0.65	15.61	±0.47	8.79	±0.32	χχχ λλλ δδδδ εεεε ωωωω φφφφ
	60	16.06	±1.37	16.80	±0.87	13.91	±1.00	15.67	0.80	18.00	±0.73	16.53	±0.57	7.75	±0.32	δδδδ εεεε χχχχ λλλλ ωωωω φφφφ
	120	13.07	±1.45	12.77	±0.67	11.73	±0.91	12.29	0.90	14.95	±1.10	13.87	±0.62	8.80	±1.55	δδ εε ωωωω φφφφ
	240	10.65	±1.81	8.56	±0.44	8.50	±0.27	8.83	0.57	10.23	±1.12	10.24	±0.53	7.64	±0.13	φ
6th OGTT (29 WOT)	0	8.10	±0.31	7.63	±0.29	7.58	±0.35	6.99	0.20	7.94	±0.18	7.55	±0.17	6.37	±0.19	-
	15	15.14	±1.31	14.61	±0.42	12.83	±0.53	13.58	0.62	15.22	±1.05	14.79	±0.35	10.02	±0.17	εε δδδ ωωω φφφφ
	30	16.10	±1.34	15.64	±0.60	13.49	±0.73	15.40	1.09	17.45	±1.09	16.64	±0.43	9.02	±0.39	χχ δδδδ εεεε λλλλ ωωωω φφφφ
	60	16.30	±1.69	16.23	±0.72	14.53	±1.15	15.76	0.85	18.51	±1.35	17.12	±0.47	7.85	±0.25	δδδδ εεεε χχχχ λλλλ ωωωω φφφφ
	120	15.14	±1.68	14.07	±0.97	11.95	±0.86	13.63	0.66	16.04	±1.27	15.28	±0.67	7.36	±0.18	χχ δδδδ εεεε λλλλ ωωωω φφφφ
	240	12.27	±2.14	9.42	±0.71	8.15	±0.48	8.81	0.59	10.35	±1.07	10.65	±0.56	7.48	±0.22	φφ δδδ
7th OGTT (32 WOT)	0	8.98	±0.56	7.66	±0.33	7.76	±0.24	7.84	0.28	9.11	±0.65	7.96	±0.16	6.89	±0.13	-
	15	16.50	±1.35	15.15	±0.62	13.66	±0.42	13.79	0.47	16.04	±1.36	15.22	±0.41	10.31	±0.19	εε ωωω δδδ φφφφ
	30	17.98	±1.52	16.70	±0.77	14.59	±0.75	15.98	0.38	17.74	±1.62	16.79	±0.43	8.99	±0.50	χχ δδδδ ωωωω λλλλ εεεε φφφφ
	60	18.29	±1.74	17.84	±0.93	15.34	±1.19	17.59	0.76	17.29	±1.12	17.84	±0.60	7.76	±0.32	δδδδ χχχχ ωωωω λλλλ εεεε φφφφ
	120	15.93	±2.17	15.47	±0.95	12.54	±1.25	16.25	0.68	15.76	±1.81	16.02	±0.70	7.61	±0.21	χχ δδδδ ωωωω λλλλ εεεε φφφφ
	240	12.55	±2.45	9.68	±1.05	8.43	±0.40	9.71	0.93	10.60	±1.59	11.61	±0.71	7.64	±0.16	φφ δδδ

Table 8: Means and standard error of means of the glucose levels (mmol/l) of all groups at the different timepoints (0, 15, 30, 60, 120, 240 minutes) in all OGTTs. See legend of symbols for significant differences. WOT = weeks of treatment.

Weeks of treatment	*Tg 0.3mg/kg (mmol/l/min)*		*Tg 1mg/kg (mmol/l/min)*		*Tg 3mg/kg (mmol/l/min)*		*Tg 10mg/kg (mmol/l/min)*		*Tg LALA-PG (mmol/l/min)*		*Tg PBS (mmol/l/min)*		*WT PBS (mmol/l/min)*		*Significances*
4	2431	±97	2291	±77	2136	±66	2234	±63	2405	±52	2409	±62	2051	±40	δ ω φφ
9	2541	±106	2596	±171	2256	±55	2375	±66	2664	±127	2596	±78	2019	±85	δ ε ωω φφφ
14	2954	±186	2719	±143	2441	±140	2657	±92	2987	±147	2914	±85	1947	±27	λλ εεε δδδδ ωωωω φφφφ
19	2865	±183	2793	±147	2466	±176	2725	±83	3056	±157	2975	±93	2098	±201	δ ε ωωω φφφφ
25	3165	±324	3042	±99	2748	±133	2910	±136	3432	±200	3229	±116	2002	±148	εε δδδ ωωωω φφφφ
29	3483	±389	3190	±150	2771	±145	3067	±120	3578	±262	3438	±109	1865	±38	δδδδ ωωωω εεεε φφφφ
32	3729	±456	3436	±187	2916	±189	3462	±124	3541	±349	3607	±136	1902	±44	εεε δδδδ ωωωω φφφφ

Table 9: Means and standard error of means of the glucose AUC (mmol/l/min) of different treatment groups in all OGTTs. See legend of symbols for significant differences.

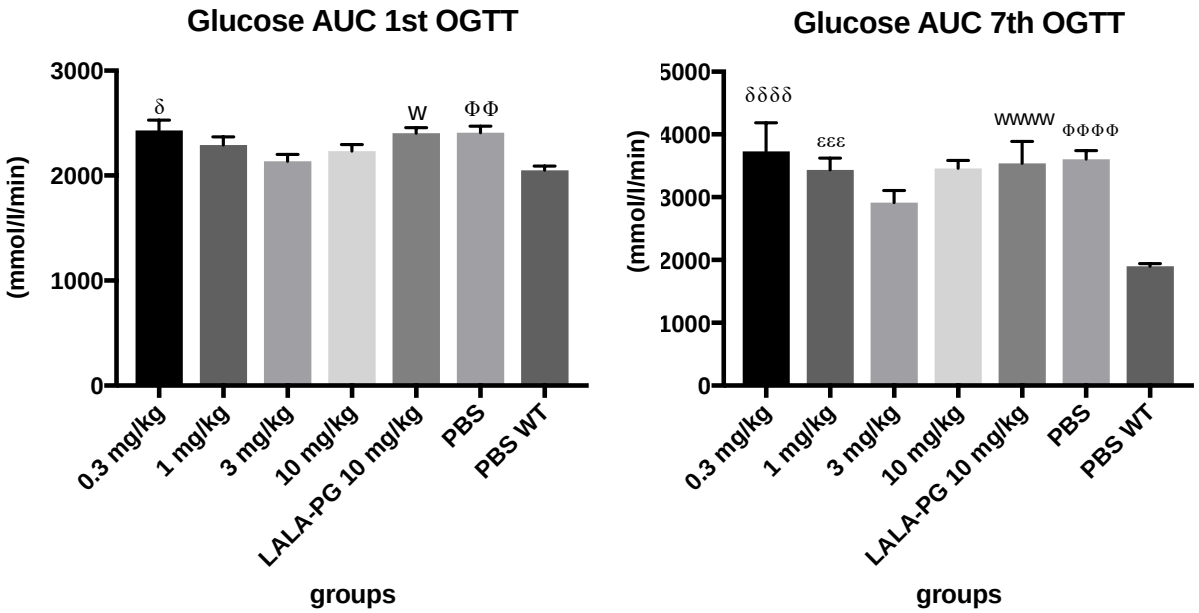


Figure 16: Comparison of the glucose AUC (mmol/l/min) of groups in the baseline OGTT and 7th OGTT. See legend of symbols for significant differences.

Insulin response

The insulin response was measured during the baseline OGTT and the 1st, 2nd, 5th and 7th OGTT. In the baseline OGTT, the Tg rats already had significantly lower insulin levels in the 30-, 120- and 240-minute timepoints. Over all OGTTs, various Tg groups showed a highly significant decrease in insulin compared to WT in mostly the 15-, 30-, 60- and 240-minute time points. In the first OGTT, all groups except for the 1 and 10mg/kg had significantly lower insulin levels in the 30- and 120-minute timepoints. Compared to this, all the Tg groups differed significantly from the WT PBS only in the 15-minute timepoint in the 2nd OGTT. In the 5th OGTT however, all Tg groups had significantly lower insulin levels than the WT PBS in the 15-, 30-, 60- and 240-minute timepoint. Less of a difference was observed in the 7th OGTT, where all Tg groups had significantly lower insulin levels compared to the WT PBS in the 15- and 30-minute time points; and all Tg groups except for the 1mg/kg Tg group in the 240-minute timepoint. Throughout all the OGTTs, there were also individual points in time when only the LALA-PG or Tg PBS groups had significantly decreased insulin level compared to WT PBS. No significant difference in insulin response was seen at any the time point between the transgenic treatment groups. (Table 10).

Results

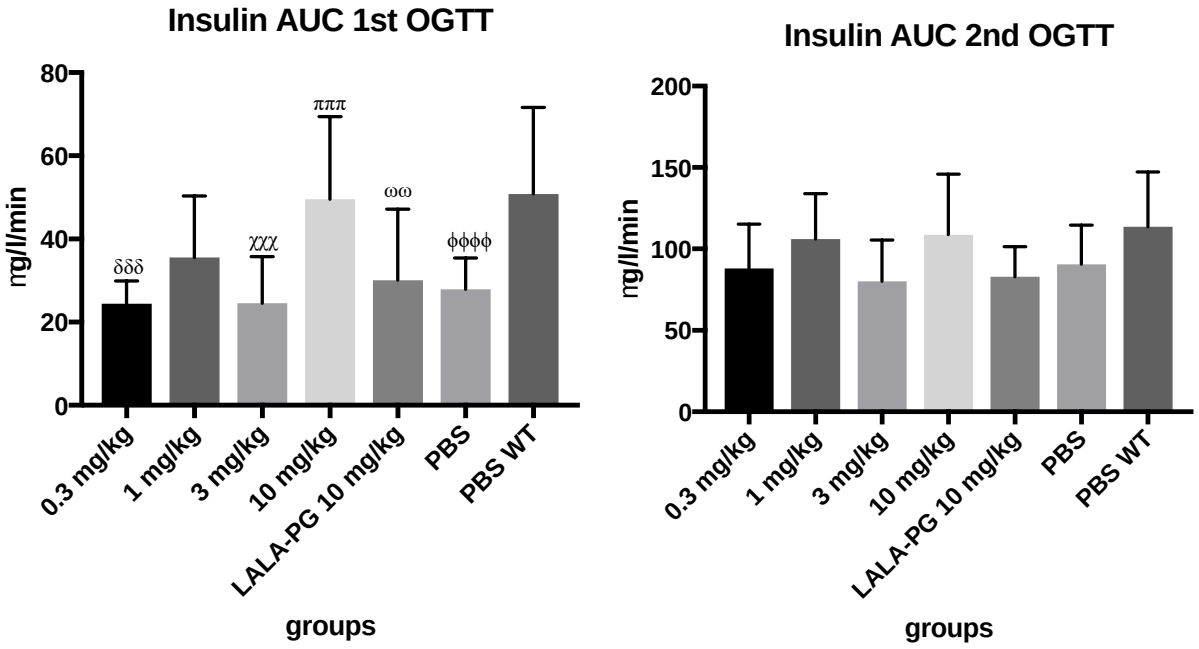
	(min)	*Tg 0.3mg/kg (µg/l)*		*Tg 1mg/kg (µg/l)*		*Tg 3mg/kg (µg/l)*		*Tg 10mg/kg (µg/l)*		*Tg LALA-PG (µg/l)*		*Tg PBS (µg/l)*		*WT PBS*		*Significances*
	0	0.13	±0.00	0.16	±0.01	0.12	±0.01	0.15	±0.01	0.14	±0.01	0.15	±0.01	0.16	±0.01	-
	15	0.13	±0.02	0.18	±0.03	0.09	±0.01	0.14	±0.02	0.12	±0.01	0.13	±0.01	0.19	±0.03	-
Baseline OGTT	30	0.10	±0.01	0.16±	±0.02	0.10	±0.02	0.14	±0.01	0.13	±0.02	0.13	±0.01	0.24	±0.06	λ ωω δδδ χχχ φφφφ
(-1 WOT)	60	0.16	±0.02	0.18	±0.04	0.10	±0.01	0.13	±0.01	0.12	±0.01	0.17	±0.02	0.14	±0.02	-
	120	0.13	±0.01	0.21	±0.03	0.13	±0.01	0.18	±0.01	0.16	±0.02	0.17	±0.01	0.25	±0.06	ω φ δδ χχ
	240	0.13	±0.01	0.17	±0.01	0.11	±0.01	0.15	±0.01	0.11	±0.01	0.16	±0.01	0.22	±0.03	χ ωω
	0	0.08	±0.00	0.13	±0.01	0.08	±0.01	0.11	±0.02	0.10	±0.01	0.10	±0.01	0.22	±0.04	-
	15	0.13	±0.02	0.16	±0.02	0.14	±0.02	0.21	±0.04	0.17	±0.02	0.15	±0.02	0.31	±0.04	-
1^{st} OGTT	30	0.02	±0.01	0.08	±0.01	0.04	±0.01	0.08	±0.02	0.04	±0.01	0.05	±0.01	0.22	±0.03	λ ωω δδδ χχχ φφφφ
(4 WOT)	60	0.10	±0.02	0.13	±0.03	0.07	±0.02	0.22	±0.06	0.10	±0.02	0.11	±0.01	0.23	±0.03	-
	120	0.12	±0.01	0.20	±0.03	0.14	±0.02	0.24	±0.03	0.15	±0.04	0.15	±0.01	0.22	±0.03	ω φ δδ χχ
	240	0.10	±0.01	0.11	±0.01	0.09	±0.01	0.20	±0.03	0.13	±0.02	0.09	±0.01	0.15	±0.02	χ ωω
	0	0.25	±0.01	0.29	±0.02	0.25	±0.02	0.30	±0.01	0.26	±0.01	0.26	±0.01	0.43	±0.06	φ
	15	0.30	±0.04	0.36	±0.05	0.38	±0.12	0.48	±0.08	0.31	±0.02	0.32	±0.02	0.71	±0.11	λ δδδδ χχχχ ωωωω εεεε φφφφ
2^{nd} OGTT	30	0.24	±0.03	0.35	±0.05	0.27	±0.07	0.32	±0.05	0.22	±0.02	0.28	±0.02	0.41	±0.03	λ
(9 WOT)	60	0.47	±0.04	0.57	±0.07	0.47	±0.03	0.60	±0.09	0.50	±0.03	0.50	±0.03	0.48	±0.08	-
	120	0.38	±0.05	0.46	±0.04	0.31	±0.04	0.47	±0.07	0.34	±0.03	0.40	±0.03	0.41	±0.04	-
	240	0.34	±0.03	0.40	±0.03	0.28	±0.04	0.38	±0.03	0.31	±0.03	0.32	±0.01	0.53	±0.06	χ ω φφ
	0	0.22	±0.02	0.29	±0.02	0.20	±0.02	0.24	±0.02	0.24	±0.02	0.23	±0.01	0.38	±0.04	-
	15	0.36	±0.04	0.42	±0.03	0.35	±0.02	0.35	±0.04	0.36	±0.05	0.43	±0.05	1.53	±0.25	δδδδ χχχχ ωωωω λλλλ εεεε φφφφ
5^{th} OGTT	30	0.33	±0.04	0.38	±0.03	0.28	±0.02	0.30	±0.01	0.33	±0.04	0.33	±0.02	0.87	±0.12	δδδδ χχχχ ωωωω λλλλ εεεε φφφφ
(25 WOT)	60	0.42	±0.04	0.64	±0.09	0.38	±0.03	0.47	±0.08	0.43	±0.04	0.44	±0.02	0.96	±0.12	ε λλλ δδδδ χχχχ ωωωω φφφφ
	120	0.42	±0.04	0.59	±0.09	0.42	±0.06	0.53	±0.08	0.44	±0.06	0.46	±0.02	0.70	±0.08	φ
	240	0.53	±0.09	0.58	±0.06	0.45	±0.05	0.47	±0.06	0.47	±0.05	0.52	±0.03	0.97	±0.08	εε λλλ δδδ χχχχ ωωωω φφφφ
	0	0.42	±0.14	0.38	±0.04	0.28	±0.01	0.30	±0.02	0.29	±0.03	0.28	±0.01	0.45	±0.04	-
	15	0.62	±0.18	0.58	±0.08	0.45	±0.06	0.45	±0.05	0.37	±0.03	0.42	±0.03	1.76	±0.23	δδδδ χχχχ ωωωω λλλλ εεεε φφφφ
7^{th} OGTT	30	0.39	±0.10	0.33	±0.04	0.28	±0.05	0.30	±0.04	0.21	±0.02	0.28	±0.02	0.86	±0.16	δδ χχχ λλλ εεε ωωωω φφφφ
(32 WOT)	60	0.59	±0.18	0.57	±0.07	0.52	±0.0	0.52	±0.06	0.39	±0.04	0.42	±0.02	0.91	±0.12	ωω φφφφ
	120	0.64	±0.17	0.70	±0.05	0.61	±0.04	0.68	±0.09	0.56	±0.10	0.55	±0.04	0.91	±0.14	φφ
	240	0.65	±0.16	0.75	±0.13	0.55	±0.07	0.60	±0.09	0.58	±0.08	0.52	±0.03	1.07	±0.14	δ λ χχ ωω φφφφ

Table 10: Means and standard error of means of insulin levels (µg/l) of groups at the different points in time (0, 15, 30, 60, 120, 240 minutes) in all OGTTs. See legend of symbols for significant differences. WOT = weeks of treatment.

At the baseline OGTT, the insulin AUC already differed significantly between the WT and Tg rats. In the 1st OGTT, all Tg groups except for the 10 and 1mg/kg groups had a smaller insulin AUC compared to the WT PBS group. In the 5th and 7th OGTT, a decrease in the insulin AUC of all Tg groups was observed compared to the WT PBS group. In the 1st OGTT, the 10mg/kg group had a significantly larger AUC than the 0.3 and 3mg/kg group as well as the LALA-PG and Tg PBS groups. Apart from that, no significant difference in insulin response was seen in any time points or in the AUC between the Tg groups in any of the OGTTs (Table 11, Figure 17).

WOT	*Tg 0.3mg/kg (µg/l/min)*		*Tg 1mg/kg (µg/l/min)*		*Tg 3mg/kg (µg/l/min)*		*Tg 10mg/kg (µg/l/min)*		*Tg LALA-PG (µg/l/min)*		*Tg PBS (µg/l/min)*		*WT PBS (µg/l/min)*		*Significances*
4	24.45	±1.72	35.57	±4.67	24.57	±3.96	49.57	±7.03	30.11	±5.40	27.92	±1.33	50.82	±5.56	ωω δδδ χχχ πππ ϕϕϕϕ
9	88.06	±8.62	106.10	±8.83	80.08	±8.97	108.70	±13.18	82.90	±5.88	90.64	±4.24	113.50	±8.99	-
25	102.5	±10.56	133.80	±11.83	95.19	±8.18	110.80	±11.81	101.40	±10.33	107.40	±4.25	210.20	±15.76	δδδδ χχχχ ωωωω λλλλ εεεε ϕϕϕϕ
32	144.4	±38.50	152.70	±15.36	125.9	±9.15	136.10	±13.86	115.30	±15.87	114.10	±5.37	235.30	±27.89	δ λ ε χχ ωωω ϕϕϕϕ

Table 11: Means and standard error of means of AUC (area under curve) of all groups in the 1st, 2nd, 5th and 7th OGTT. See legend of symbols for significant differences.



Results

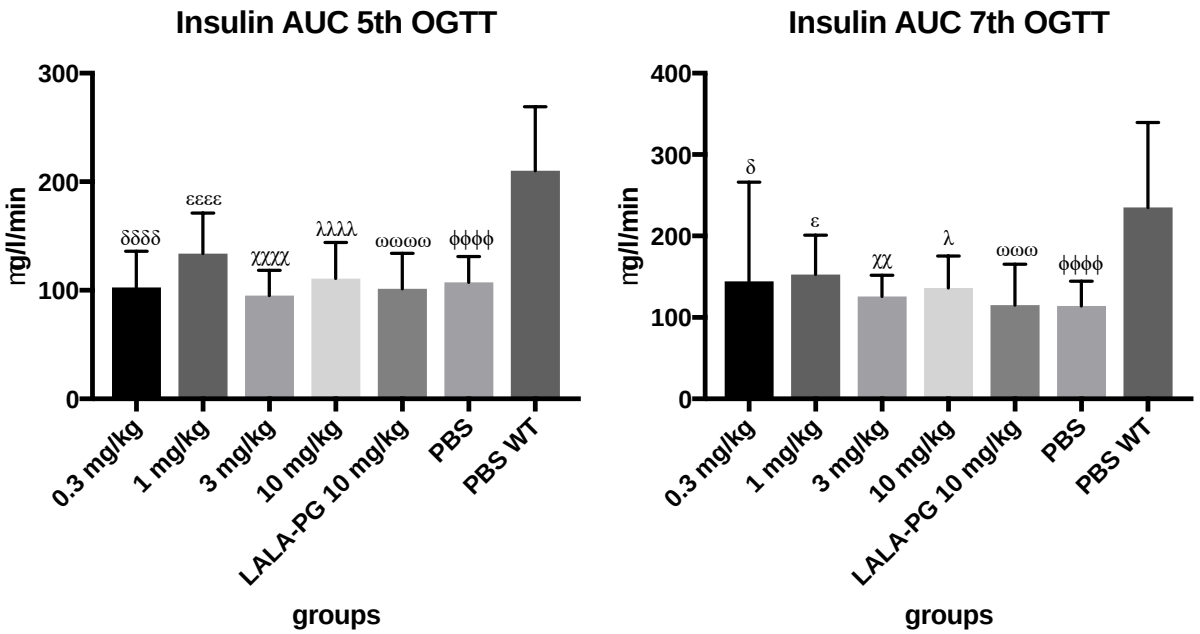


Figure 17: Comparison of the insulin AUC (area under curve) in µg/l/min of all groups in the 1st, 2nd, 5th and 7th OGTT. See legend of symbols for significant differences.

Fasting plasma IAPP levels

Fasting plasma IAPP levels were measured at the baseline OGTT as well as the 2nd, 4th, 5th and 7th OGTT. The levels of all groups stayed consistent except for the Tg LALA-PG and 10mg/kg groups, which had increased plasma IAPP levels in some of the OGTTs (Figure 18). When comparing the IAPP levels of the WT to the Tg rats, the WT PBS group had significantly decreased IAPP levels compared to the 10mg/kg group in the 2nd and 4th OGTT, as well as compared to the LALA-PG group in the 7th OGTT. Within the Tg rats, the 10mg/kg had significantly higher IAPP levels than the Tg PBS group in the 2nd OGTT. Also, the 10mg/kg group had significantly higher IAPP levels than the Tg PBS, 0.3, 1 and 3mg/kg groups in the 4th OGTT. In the 7th OGTT, the IAPP levels of the Tg LALA-PG group was significantly higher than that of the Tg PBS, 1 and 3mg/kg groups (Figure 18, Table 12).

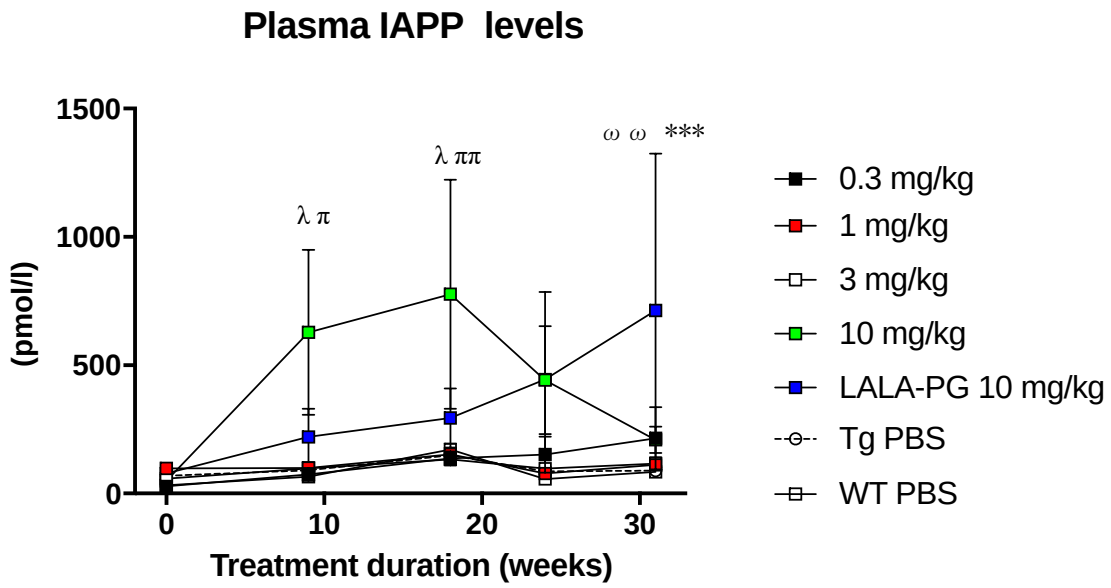


Figure 18: Course of plasma IAPP (pmol/l) levels of all groups over 32 weeks. See legend of symbols for significant differences.

OGTT	*Tg 0.3mg/kg (pmol/l)*		*Tg 1mg/kg (pmol/l)*		*Tg 3mg/kg (pmol/l)*		*Tg 10mg/kg (pmol/l)*		*Tg LALA-PG (pmol/l)*		*Tg PBS (pmol/l)*		*WT (pmol/l)*		*Significances*
Baseline	28.3	±9.5	98.0	±16.1	57.5	±19.8	49.6	±18.0	78.6	±23.0	69.4	±12.0	32.1	±7.5	-
2nd	74.4	±25.5	99.3	±14.9	97.7	±24.8	627.7	±321.3	220.8	±110.7	90.7	±11.0	65.3	±10.0	λ π
4th	137.5	±28.7	152.7	±22.1	132.8	±22.0	776.4	±447.0	294.6	±114.7	149.4	±16.0	172.2	±23.2	λ π π
5th	151.8	±69.6	77.5	±4.1	96.7	±15.0	442.3	±210.1	444.0	±341.3	86.5	±8.5	56.8	±12.3	-
7th	215.2	±121.3	111.6	±19.6	117.4	±26.1	210.0	±51.6	712.8	±611.5	88.9	±8.4	84.8	±11.6	ω ω ***

Table 12: Means and standard error of means of the baseline IAPP levels (pmol/l) of all groups before the baseline, 2nd, 4th, 5th and 7th OGTT. See legend of symbols for significant differences.

5.4. Pharmacokinetics and –dynamics (PK/PD)

Due to a lack of progression of the diabetic phenotype in the expected time frame, this part of the study was postponed. This is discussed in further details in the general Discussion.

5.5. Target engagement

After a long trial regarding target engagement in fixed rat pancreas, it was discovered that this could only be achieved in unfixed frozen pancreas. This is most likely because the hNI-203.26C11-bound oligomeric hIAPP species are sensitive to fixation and do not preserve their native conformation recognized by hNI-203.26C11.
Based on this finding, the following protocol was established: 3 days after a single injection of hNI-203.26C11 antibody (30 mg/kg) or vehicle in HIP rats, the rats were sacrificed and the pancreas was harvested. The unfixed, frozen pancreas sections were incubated with a fluorescently-labeled anti-human antibody. This was combined with insulin counterstaining. Together, this resulted in the visualization of diffuse extracellular IAPP aggregates around insulin-producing β-cells. As a control, these were compared to the pancreata of HIP rats after a single injection of vehicle following the same protocol (Figure 19).

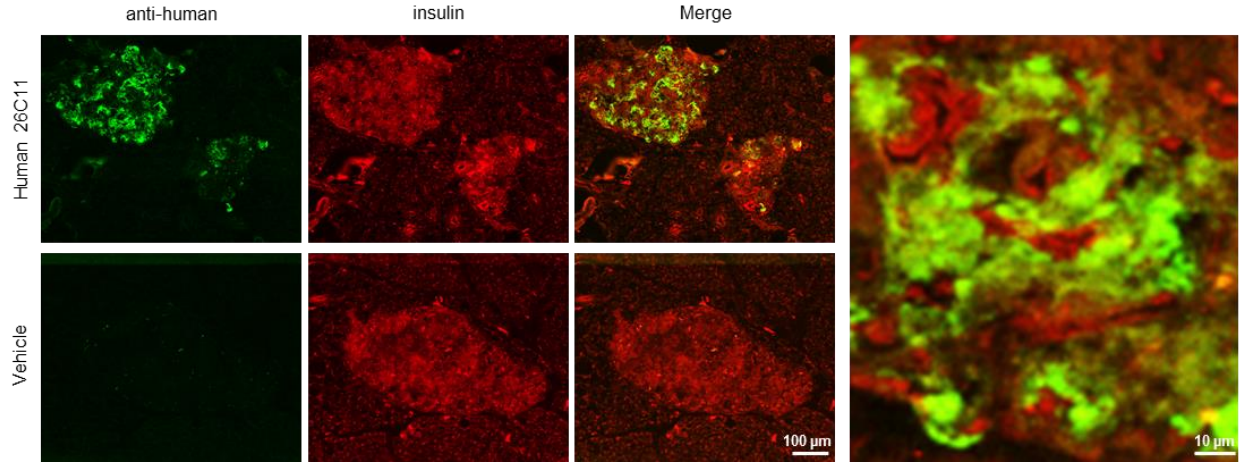


Figure 19: Pancreas immunostaining for insulin (1° guinea pig anti-insulin AB and 2° TRITC-labeled antibody) and hNI-203.26C11 with a fluorescent 2° anti-human antibody (Neurimmune AG) after a single injection of hNI-203.26C11 or vehicle in HIP rats. Serving as a control, the fluorescently labeled hNI-203.26C11 occurs specifically in rats treated with hNI-203.26C11, but not in those injected with PBS. The fluorescently labeled insulin occurs to a similar extent in both rats. When merging these two images, the hNI-203.26C11 antibody areas overlap with insulin-rich areas in the rats injected with hNI-203.26C11. This indicates that these diffuse extracellular IAPP oligomers are surrounding insulin-producing β-cells.

6. Discussion

T2D is characterized by insulin resistance, defective insulin secretion, hyperglycemia, loss of β-cell mass and function as well as micro- and macrovascular complications (Stumvoll et al. 2005; Despa et al. 2012). This condition is initially caused by insulin resistance and leads to an activation of several compensation mechanisms, namely an initial increase in β-cell mass and insulin secretion. This increased insulin secretion is accompanied by increased IAPP secretion, as both are stored and co-secreted in vesicles of β-cells. In turn, increased IAPP secretion facilitates the aggregation of pathogenic IAPP. In this process cytotoxic IAPP oligomers are formed, which induce oxidative stress, islet inflammation and β-cell apoptosis (Abedini et al. 2016). So far, there are no therapies that aim to prevent the process of IAPP aggregation or that target these cytotoxic oligomers specifically. Therefore, the novel approach of antibody therapy tested in this study has great potential. Based on this, the lead antibody NI-203.26C11 was selected for in vivo testing after the initial identification and production of antibody candidates with high selectivity and affinity for toxic hIAPP oligomers, but not monomeric IAPP or large extracellular IAPP deposits.

Animal model

For the animal model, rats transgenic for hIAPP expression (HIP rats) were chosen due to the pathophysiologic process of hIAPP aggregation shared with humans suffering from T2D. As in diabetic humans, middle-age diabetes develops spontaneously in HIP rats and progresses with age. The islet pathology corresponds to that in humans and ultimately results in hyperglycemia, insulin resistance and decreased β-cell mass (Matveyenko and Butler 2006). Further, this model does not require severe obesity for the development of diabetes, as HIP rats develop diabetes spontaneously and independent from obesity. Based on this, the HIP model mimics many facets of the metabolic syndrome, but in particular IAPP aggregation, which is not present in other frequently used rodent models.

In mouse models, mice transgenic for hIAPP expression were crossed with obese mouse models, for example ob/ob mice (Höppener et al. 1999), (a(vy)/a) mice (Soeller et al. 1998; Geisler et al. 2002; Butler et al. 2003). This resulted in heterozygous mouse models that reflect both the pathogenic role of hIAPP expression as well as the pathogenic aspect of lipotoxicity. Up to this time point, no such rat model had been developed or is available for purchase. HIP rats were also used as animal model in previous studies conducted by our group in collaboration with Neurimmune AG.

Observations of previous studies

After a 30-week treatment with chimeric rNI-203.26C11 (3mg/kg) in an initial study, first beneficial effects were observed in HIP rats, resulting in improved glucose tolerance, decreased fasting glucose, increased plasma insulin levels and normalized body weight gain (M. Osto 2014, 2015). These changes indicate an overall preservation of β-cell function. In a further study, the efficacy of three different dosages (1, 3 and 10mg/kg) was examined over a period of 40 weeks (Hugentobler 2017). In that study, the protective effect on β-cells observed beforehand was reconfirmed, as all three dosages slowed the progression of diabetes, yet without a clear dose-response relationship. Additionally, WT rats treated with the same dosages showed no unspecific side effects.

Aims of the present study

In the present study, the three main aims were the following: to investigate the long- and short-term efficacy of four different dosages of rNI-203.26C11, to examine the mechanisms of action

more closely and to develop a method of visualization and quantification of rNI-203.26C11 mediated hIAPP clearance.

6.1. Beneficial effects of long-term treatment

The first aim of this study was to investigate the long-term effect of four different dosages of rNI-203.26C11 (0.3, 1, 3, 10mg/kg) on BW gain, β-cell function and plasma IAPP levels in glucose intolerant HIP rats. After 32 weeks of treatment, a decrease in BW was observed in the PBS, 0.3mg/kg and LALA-PG (an inert version of the antibody rNI-203.26C11) treated Tg groups compared to the WT PBS. This was paralleled by an increase in fasting glucose levels and glucose AUC. However, these observations were not made concerning the 1, 3 and 10mg/kg rNI-203.26C11 treated groups. This indicates an early protective effect on β-cell viability and a slowing of the progression of T2D in dosages ranging between 1 and 10mg/kg, although not in a strictly dose dependent manner.

Effect on BW

Up to 45 weeks of age, a general BW gain was observed in the antibody-treated Tg groups ranging from a dose of 1 to 10mg/kg as well as the WT PBS. No plateau was reached up to this point. However, a significant decrease in BW gain occurred in the 0.3mg/kg, LALA-PG and Tg PBS treated groups compared to the WT PBS group from 42 weeks of age onwards. This decrease in both PBS and 0.3mg/kg treated Tg groups progressed to be highly significant at 43, respectively 44, weeks of age. BW loss is thought to reflect at least in part energy loss by glucosuria due to insulin deficiency. Based on that, this observation indicates that a long-term treatment with the antibody NI-203.26C11 delays the development of a diabetic phenotype, but was unable to prevent it, at least at this low antibody dose. This protective effect was also observed in our previous studies, where the BW of the antibody treated groups ranging from a dosage of 1 to 10mg/kg remained stable compared to the Tg PBS group, which lost weight from 25 weeks of treatment (37 weeks of age) onwards (Hugentobler 2017). Nonetheless, in this study the 10mg/kg group also began to show a significant decrease in BW gain compared to the WT PBS group at 45 weeks of age. This occurrence might be the first indication of the development of a diabetic phenotype in all Tg rats regardless of their treatment.

Effect on FI

In the development of the HIP rat model, a certain variability concerning FI levels had been observed in both Tg and WT rats (Butler et al. 2004). This variability was also seen in our groups, as no consistent changes were observed between our Tg groups. Despite the overlap in values due to the inter-individual variability, the Tg rats developed insulin deficiency at 10 months of age compared to WT rats (Butler et al. 2004; Aitken et al. 2017; Matveyenko and Butler 2006). In this study, the FI levels of the Tg PBS rats showed a highly significant decrease from the 1st until the last OGTT compared to the WT rats, from an age of 16 weeks onwards. A similar situation was observed in our previous study, where a significantly decreased FI occurred when comparing the Tg PBS to the WT PBS in the 2nd OGTT and again from the 6th OGTT until sacrifice. Just as in our study, no significant differences were observed between the Tg groups (Hugentobler 2017). However, significantly lower FI values were observed in the Tg 0.3mg/kg, 3mg/kg and PBS group compared to the WT PBS group from the 1st OGTT and in the LALA-PG group from the 2nd OGTT on in this study. Out of all the groups, it seems that FI of the 1mg/kg treatment group was overall higher than that of the other Tg groups, therefore not resulting in a

significant difference compared to the WT PBS. This observation may correspond to a delayed onset of impaired β-cell function.

Effect on insulin AUC and response

The key physiological function of insulin is to respond to an increase in blood sugar levels and consequently cause a decrease in circulating glucose levels. Based on this notion, the insulin response is thought to reflect β-cell function more accurately than the glucose response. This notion is confirmed by the observation that a defective insulin response precedes hyperglycemia in both HIP rats as well as humans with T2D (Matveyenko and Butler 2006; Boden et al. 1968; van Haeften et al. 1998). In this study, all Tg groups had a significantly decreased insulin AUC in the 1st OGTT (except for the 10mg/kg Tg group) as well as in the 5th and 7th OGTT compared to the WT PBS. This increased insulin AUC of the 10mg/kg group compared to the Tg PBS was also observed in the 2nd OGTT at 20 weeks of age in a previous study (Hugentobler 2017). Interestingly, no significant differences occurred between the groups in the 2nd OGTT in the current study. In the 1st OGTT, the insulin AUC of the 10mg/kg treatment group was increased in comparison to all Tg groups except for 1mg/kg Tg group. No significant difference between Tg groups occurred in the following OGTTs. These significant differences between the Tg groups and the WT PBS were also reflected in the insulin levels at the different points in time of the OGTT, while no significant differences were observed between the Tg groups.

Overall, this suggests that all the Tg groups developed an impaired insulin response to some degree between the age of 22 and 27 weeks of age independent of treatment or dosage. Therefore, the long-term treatment with rNI-203.26C11 was not able to lead to a significant reduction in the progression of glucose intolerance in the current study.

Effect on FG

In previous papers, a progressive increase in FG was observed in Tg HIP rats. This increase is thought to reflect progressive β-cell loss. Overall, we observed a highly significant increase in FG levels of the 0.3mg/kg, LALA-PG and Tg PBS group compared to the WT PBS from the 4th OGTT (32 weeks of age, 19 weeks of treatment, respectively). Compared to this, no consistently higher FG levels were observed in the Tg 1mg/kg, 3mg/kg and 10mg/kg treatment groups compared to the WT PBS. This also indicates that long-term treatment with NI-203.26C11 has a protective effect of on β-cell function. In the previous study concerning the efficacy of three dosages of rNI-203.26C11 in long-term therapy (Hugentobler 2017), significantly lower FG levels were observed in the Tg 3mg/kg group (5th and 6th OGTT), in the 1mg/kg group (8th to 10th OGTT) as well as in the 10mg/kg group (10th OGTT) compared to the Tg PBS group. Concurrent with our study, no significant differences were observed between the different dosage groups. The β-cell content of the rats in our experiment has yet to be histologically determined.

Effect on glucose AUC and response

The cytotoxic effect of hIAPP oligomers on β-cells leads to a decrease in β-cell mass and function. This leads to impaired insulin secretion, which in turn results in glucose intolerance (Haataja et al. 2008; Jurgens et al. 2011; Butler et al. 2004). When examining the glucose tolerance in the OGTTs, less of an impaired glucose response was observed in the 3 and 10mg/kg treatment groups compared to the other Tg groups. Compared to the WT PBS, the 0.3mg/kg, LALA-PG and Tg PBS groups had significantly increased glucose AUCs in all OGTTs, while the

1mg/kg group did so from the 2nd OGTT onwards. In contrast, the glucose AUC of the 3mg/kg group was never increased significantly, and that of the 10mg/kg group was increased only in the 3rd OGTT compared to the WT PBS. In our previous study (Hugentobler 2017), an overall lower glucose AUC was observed in the 1 and 10mg/kg Tg treatment groups from the 2nd until the 8th OGTT (20 to 46 weeks of age). In the present study, however, no consistent significant differences were observed within the Tg treatment groups. From the 1st to the 4th OGTT, the glucose levels of the 3mg/kg group were significantly lower than those of the Tg PBS group in the 30- or 60-minute time points, or both. Also, except for the 7th OGTT, a significant decrease in glucose levels was observed in the 3mg/kg group compared to the LALA-PG 10mg/kg group in at the 60- and/or 120-minute time points in all OGTTs. In the last OGTT, no significant difference in glucose tolerance or in glucose AUC was seen at any time point between the Tg groups. Compared to the WT rats, all Tg groups showed significantly higher glucose levels in the 15-minute point, except for the 3 and 10mg/kg groups. All Tg groups had significantly higher glucose levels in the 30-, 60- and 120-minute time points. At the 240-minute point, only the 0.3mg/kg and Tg PBS groups still had elevated glucose levels compared to the WT PBS group. On one hand, these observations may reflect an impaired glucose response independent of treatment during the experiment, while it is less severe in the 3 and 10mg/kg treatment groups. On the other hand, these observations indicate an enhanced glucose response in both the 3 and 10mg/kg groups compared to the other Tg groups.

Effect of treatment with rNI-203.26C11 on plasma IAPP

Plasma IAPP levels were measured using a commercial ELISA assay detecting both monomeric human and rat IAPP. As the antibody NI-203.26C11 binds to aggregated hIAPP oligomers specifically, it is unlikely that NI-203.26C11-bound IAPP or aggregated IAPP was detected in the ELISA assay. In previous studies (Matveyenko and Butler 2006), no difference in fasting plasma IAPP level was observed between the Tg PBS and WT PBS groups from 2 to 10 months of age. When comparing fasting plasma IAPP levels, in our study both those of the 10mg/kg treated rNI-203.26C11 and the LALA-PG rNI-203.26C11 group were significantly higher than those of the Tg and WT PBS groups, but at different points in time. In the 10mg/kg rNI-203.26C11 treated Tg group, a plasma IAPP peak was observed between 22 and 32 weeks of age (in the 2nd and 4th OGTT). In a previous study, a similar phenomenon was observed (M. Osto 2014); after 18 weeks of treatment (at an age of 30 weeks), a peak in plasma hIAPP levels occurred in the 3mg/kg rNI-203.26C11 treated Tg rats but not in the Tg PBS control group. In that study those two groups were not compared to WT rats, nor to other dosages. In the 7th OGTT of our study (32 weeks of treatment, 45 weeks of age), the LALA-PG rNI-203.26C11 group (10mg/kg) had significantly increased plasma IAPP levels compared to both Tg and WT PBS treated groups. This might reflect either an increased formation and production of free IAPP or an increased IAPP elimination from the pancreas into the blood. Both would be a further indication of the beneficial effect of antibody treatment with rNI-203.26C11.

Effect of rNI-203.26C11 dose on plasma IAPP levels

In the first instance, it appears that a correlation exists between the increased plasma IAPP level and the administration of a higher dosage of antibody, as an this was only observed in both the 10mg/kg rNI-203.26C11 and LALA-PG rNI-203.26C11 treated groups. The underlying cause of this observation is most likely linked to further mechanisms of action other than macrophage activation. On one hand, this might be the indirect result of inhibited IAPP aggregation through catalytic dissolution(Morgan 2011)(Morgan 2011) (Solomon et al. 1996; Solomon et al. 1997;

Schenk et al. 1999), resulting in a greater amount of free IAPP monomers. On the other hand, this might also be due to an increased clearance of monomeric soluble hIAPP or disassembly of hIAPP aggregates which may be detected by the assay, combined with increased IAPP secretion resulting from improved β-cell function. Overall, this observation supports the theory that more than one mechanism of action is involved in the inhibition of aggregation and hIAPP clearance. Despite the similar binding profiles of the inert LALA-PG rNI-203.26C11 compared to rNI-203.26C11, a timewise difference in the occurrence of the elevated plasma hIAPP values was observed. This might reflect the importance of other mechanisms of action, leading to a delay in hIAPP clearance in the rats treated with the inert LALA-PG NI-203.26C11 compared to the 10mg/kg NI-203.26C11 treated rats. Based on this, this observation is most likely linked to efficacy. In the development of antibody therapy in AD, plasma Aβ levels were also examined over a time span of 500 hours following a single dose application of aducanumab or 3D6 (Sevigny et al. 2016). A single dose of aducanumab, which does not bind to Aβ monomers, did not result in increased Aβ concentrations in the plasma or brain, even after repeated dosing. In comparison, a single dose administration of 3D6, a murine bapineuzumab precursor antibody with the ability to bind to Aβ monomers, resulted in a transient plasma Aβ spike 100h post-dosing. Therefore, it was assumed that there is a correlation between the ability to bind soluble Aβ and the observed plasma Aβ peak. One possible explanation to this observation is the peripheral sink mechanism, which postulates an Aβ clearance by the binding of free circulating antibodies to free Aβ (DeMattos et al. 2001). As our antibody NI-203.26C11 mostly binds specifically to pathogenic hIAPP oligomers and not to physiological monomers, a similar outcome as in aducanumab was expected, namely no increase in plasma IAPP levels. As an increase did indeed occur between 22 and 32 weeks of treatment, there may possibly be another, yet unknown long-term mechanism apart from the peripheral sink mechanism, which was not observed in the development of aducanumab.

6.2. Variability in the HIP rat model

Over the course of our previous studies, a variable diabetic phenotype of the transgenic HIP rats was observed. A delay in the progression of the diabetic phenotype was already observed in some of our previous studies. In our present study, there was a slow progression of the diabetic phenotype up to 45 weeks of age (approx. 10 months). These rats were obtained from Charles River, where they had been stored as cryopreserved embryos and then revitalized. As in many other lines, these animals descend from a very narrow breeding base. In HIP rats of the line 9, the midlife onset of the diabetic phenotype was described to occur from an age of 5 to 10 months of age, which coincides with a β-cell loss of 70-80%. This diabetic phenotype is characterized by progressive polyuria and polydipsia (PU/PD), a decrease in body weight of up to 20% compared to the WT from 5 months of age on, as well as impaired fasting glucose (Butler et al. 2004; Matveyenko and Butler 2006).

Variability concerning PU/PD

In our previous study, PU/PD and weight loss were observed in Tg PBS rats from 37 weeks of age. So far, this does not match our observations in the current study, where no generalized PU/PD or BW loss was seen up to an age of 45 weeks.

Variability concerning BW loss

In three individual rats of different treatment groups (3 and 10mg/kg, Tg PBS), a diabetic phenotype paired with severe BW loss was indeed observed at about 43 weeks of age. However,

on average, an overall BW gain was observed in all groups up to the age of 45 weeks, and no plateau was reached up to this point. The mean BW of the Tg PBS rats was significantly decreased compared to that of the WT PBS rats from 14 weeks of age. At 45 weeks (10 months) of age, our Tg PBS rats weighed 13.5% less that the WT rats. In comparison, transgenic HIP rats weighed approximately 20% less than WT rats from 5 months on (Butler et al. 2004). In a previous study (Hugentobler 2017), a weekly decrease in BW of approximately 0.5% was observed in the Tg PBS rats from 37 weeks of age, which progressed to a total loss of around 8% by the end of the study at 53 weeks of age. The smaller difference in mean BW observed in our Tg PBS rats compared to the WT PBS rats as well as the absence of weight loss of the Tg PBS groups might be indicative of a delay in the development of the diabetic phenotype.

Variability concerning impaired FG

While developing the HIP rat model, an impaired FG was observed from 5 months of age (7.1mmol/l), which progressed to 10.3mmol/l at 10 months of age (Butler et al. 2004; Matveyenko and Butler 2006). Subsequently, a further increase in FG was observed from 10mmol/l (10 months of age) up to about 15mmol/l (18 months of age) (Butler et al. 2004). In one of our previous dose response studies (Hugentobler 2017), three dosage groups (1, 3, 10mg/kg) were compared to Tg and WT PBS groups over the course of 41 weeks of treatment. In the Tg PBS group, mean FG levels of 7.8mmol/l were measured at approximately 5 months of age (20 weeks of age) and 9.4mmol/l at approximately 10 months of age (30 weeks of age). While a significant difference in FG occurred early on when comparing the Tg to the WT PBS groups (from 12 weeks of age), differences within the Tg groups were observed from 26 weeks of age onwards. In the present study in comparison, no significant difference in FG was observed when comparing the Tg PBS group to the WT PBS group until 32 weeks of age (4th OGTT). A significant increase in the FG was observed in the 0.3mg/kg, LALA-PG treatment groups compared to the WT PBS from the 4th to the 7th OGTT (32 until 45 weeks of age), but no consistent significant changes were observed between the treatment groups. While the mean FG value of the Tg PBS rats was 8.3mmol/l at 5 months of age, it decreased to a mean FG of 8.0mmol/l at 10 months of age. Further, the Tg rats had an impaired glucose response and significantly higher glucose AUC values compared to the WT rats in the baseline OGTT, and yet still no significant differences in AUC values could be observed between the Tg groups throughout all OGTTs. These observations may also reflect a delay in progression caused by the different treatments of the diabetic phenotype, as the difference in the values of the treatment groups is not pronounced enough to result in significance.

Possible underlying mechanisms

One possible explanation for this observed variability in the HIP rat model would be copy loss of the transgene. The copy number correlates with the expression of hIAPP, therefore determining the timepoint of the development of the diabetic phenotype. The idea that the time point of development of diabetes is closely associated with the transgene copy number was proven by doubling the copy number by breeding homozygous transgenic mice and rats expressing hIAPP (Janson et al. 1996). In mice, certain hemizygous mouse models did not even develop a diabetic phenotype at all (Janson et al. 1996; Couce et al. 1996; Fox et al. 1993; Koning et al. 1994; Yagui et al. 1995) Therefore, it was hypothesized that a threshold effect is involved concerning the copy number and the development of a diabetic phenotype in transgenic hIAPP mice (Butler et al. 2004). The same might apply for HIP rats. In rats, hemizygous HIP rats generally developed diabetes spontaneously at a later timepoint than their homozygous counterparts (Butler et al.

2004). In the hemizygous HIP rats (Line 9), which were used as the animal model in this study, the exact number of copies is not known and therefore cannot be determined and compared.

Summarizing remarks about the variability in the HIP rat model

The comparisons mentioned above reconfirm our observation of a delay in the progression of the development of T2D in our HIP rats. Further means to confirm a delay of progression would be to histologically analyze the β-cell content, islet amyloid and amount of bound antibody NI-203.26C11 in the islets of the different groups. Due to the lack of progression of the diabetic phenotype of the HIP rats used in this study, the planed PK/PD study was postponed. The aim of the PK/PD study was to examine if a single administration of the antibody NI-203.26C11 could restore or influence β-cell function in animals with advanced diabetes. As the study was conducted after this dissertation was written, the results will not be presented and discussed.

6.3. Macrophage activation as a key mechanism of action

The second aim of this study was to investigate the role of macrophage-mediated clearance of hIAPP aggregates and further possible mechanisms of action. To do so, an inert LALA-PG version of the antibody rNI-203.26C11 was administered to examine the importance of macrophage mediated clearance of hIAPP aggregates. This LALA-PG NI-203.26C11 has three mutations in the Fcɣ domain, which prevent interaction with Fcɣ-receptors on immune cells and immune responses (Lo et al. 2017). The treatment with the inert LALA-PG rNI-203.26C11 (10mg/kg) over 32 weeks resulted in an overall decrease in BW gain, FG and glucose AUC during the OGTT as well as an earlier decrease in FI compared to rNI-203.26C11(10mg/kg) treated group. These findings reflect an earlier onset of T2D, therefore supporting the idea that macrophage activation is a key mechanism of action of hIAPP clearance. Further, increased plasma IAPP levels were observed in both the 10mg/kg rNI-203.26C11 and 10mg/kg LALA-PG treated Tg rats, although at a later time point in the LALA-PG treated rats. This might possibly reflect a further, yet unknown long-term mechanism.

Role of macrophage activation in AD

The importance of receptor-mediated phagocytosis was also observed during the development of antibody-based approaches therapies for AD, for example in aducanumab (Sevigny et al. 2016). A previous study (Morgan 2009) gives an overview of four proposed mechanisms of inhibition of aggregation and removal of Aβ in AD and proposes that the extent of each mechanism depends on the antibody. These mechanisms include (1) catalytic dissolution, in which the process of aggregation is prevented by modifying secondary structure (Schenk et al. 1999; Solomon et al. 1997; Solomon et al. 1996; Morgan 2006); (2) MHC-2 antibody-mediated phagocytosis through macrophages (Schenk et al. 1999; Bard et al. 2000; Webster et al. 2001; Koenigsknecht-Talboo and Landreth 2005; Das et al. 2003); (3) peripheral sink mechanism, in which peripheral circulating antibodies bind to free Aβ, resulting in increased clearance (DeMattos et al. 2001); and (4) FcRn mechanism, in which the facilitated transport of antibody-Aβ complexes via the neonatal Fc receptor is proposed (Deane et al. 2005). Due to the pathological similarities between Aβ aggregates in AD and hIAPP aggregates in T2D, similar mechanisms may very well occur concerning antibody-mediated prevention of hIAPP aggregation and removal. As far as the FcRn mechanism is concerned, the expression of FcRn in interstitial and endothelial cells of the pancreas has yet to be determined.

Discussion

Role of macrophage activation in T2D and AD

In patients suffering from T2D, an immune cell infiltration in the islets was observed often, mostly consisting of macrophages (Ehses et al. 2007; Richardson et al. 2009; Kamata et al. 2014; Martino et al. 2015). On one hand, these macrophages are thought to contribute to the maintenance of islet homeostasis, thus having a protective effect. On the other hand, macrophage activation can also lead to an increase in β-cell cytotoxicity and inflammation through cytokine secretion (Eguchi and Manabe 2013; Eguchi and Nagai 2017). Therefore, there is a delicate balance between the benefits and drawbacks of macrophage activation caused by antibody therapy, with yet unknown consequences. Further, it is also unknown if antibody therapy with rNI-203.26C11 may influence the balance between M1 and M2 macrophages. For example, in antibody therapy targeting Aβ in AD, some antibodies were found to induce the release of proinflammatory cytokines. This leads to the development of vasogenic edemas as an adverse reaction, contributing to amyloid-related imaging abnormalities (ARIAs) (Sperling et al. 2012; Sperling et al. 2011; Pankiewicz and Sadowski 2017; Carlson et al. 2011; Carlson et al. 2016; Arrighi et al. 2016). In comparison to the brain, less adverse effects caused by macrophage infiltration are to be expected in the pancreas, due to its anatomical structure and physiological properties. Also, no side effects were observed due to macrophage infiltration in our previous studies. Comparing the β-cell function of rNI-203.26C11 to its inert LALA-PG version was the first step to examining the impact of macrophage activation more closely. Beforehand, we already observed that rNI-203.26C11 recruits macrophages in HIP rat islets and stimulates the uptake of hIAPP aggregates following in vitro incubation. In this study, the effect of rNI-203.26C11 (10mg/kg) on β-cell function, IAPP levels and body weight gain was compared to its' inert LALA-PG version (10mg/kg).

Influence of LALA-PG treatment in comparison to antibody-treated and PBS Tg rats

A premature onset of loss in BW gain was observed in the LALA-PG 10mg/kg group, together with the 0.3mg/kg and Tg PBS group. Further, a highly significant increase in FG was observed in the 0.3mg/kg, LALA-PG 10mg/kg and Tg PBS groups compared to the WT PBS from the 4th OGTT onwards. The Tg 0.3 and 3mg/kg groups as well as the Tg PBS group already had decreased FI levels compared to the WT PBS from the 1st OGTT on, while this was already in the LALA-PG group from the 2nd OGTT on. The glucose AUC of the 0.3mg/kg, LALA-PG and Tg PBS groups were increased significantly compared to the WT PBS in all the OGTTs. The insulin AUC of the LALA-PG group was decreased significantly compared to the WT PBS together with almost all Tg groups in the 1st OGTT and all Tg groups in the 5th as well as 7th OGTT. Further, increased fasting plasma IAPP levels were observed in the LALA-PG group compared to the WT PBS in the 7th OGTT. This increase in fasting plasma IAPP levels might reflect both higher pancreatic hIAPP content or an increased elimination of hIAPP from the pancreas into the plasma.

Conclusion of the role of macrophage activation

Overall, the values of the LALA-PG treated group concerning BW gain, FG as well as glucose and insulin AUC were found to be comparable to those of the Tg PBS and 0.3mg/kg group. A somewhat later decline in FI values was observed in the LALA-PG 10mg/kg group compared to the Tg PBS and 0.3mg/kg groups. These findings indicate that macrophage activation plays a key role of all mechanisms of action concerning the clearance of toxic hIAPP aggregates. A further step to confirm this would be to analyze the pancreatic hIAPP and macrophage content as well as

β-cell mass and bound NI-203.26C11 in the LALA-PG treated Tg group compared to the 10mg/kg treated Tg group.

6.4. Development of a method of target engagement

The third aim of this study was to develop a method for histological visualization and quantification of NI-203.26C11-mediated clearance of toxic hIAPP oligomers. This was achieved in unfixed, frozen pancreas. A diffuse extracellular occurrence of bound anti-human antibody was seen around insulin producing β-cells 3 days after a single i.p. injection (30mg/kg). This verifies that NI-203.26C11 is specific for pathogenic hIAPP oligomers and that it binds to the target within the islets. Also, bound anti-human antibody was only observed in the animals treated with hNI-203.26C11 and not in vehicle treated rats. This reconfirms the occurrence of bound hNI-203.26C11 in rats 3 days after injection of hNI-203.26C11. A further step would be the comparison to WT rats to confirm the specificity of hNI-203.26C11 to hIAPP, opposed to physiologically occurring rIAPP. Also, a combined counterstaining of bound antibody hNI-203.26C11 together with a staining for islet amyloid or physiological monomeric hIAPP would be informative. Based on the development of this method of target engagement, a correlation between target engagement and drug efficacy can be examined in the future. Further, the amount of pathogenic hIAPP bound to the anti-human antibody could be used to quantify hIAPP clearance. This novel method gives us a valuable tool to visualize and quantify rNI-203.26C11 mediated hIAPP clearance in vivo, with a broad range of application for the future.

6.5. Conclusion

In conclusion, the long-term application of the antibody NI-203.26C11 (1 to 10mg/kg) targeting toxic hIAPP oligomers in HIP rats was shown to have a protective effect on β-cell viability. When comparing the respective animals to the WT rats, NI-203.26C11 resulted in improved fasting glucose, glucose AUC during the OGTTs and prevented a loss of BW gain opposed to the Tg PBS rats. The effect of NI-203.26C11 was observed in the dose range from 1 to 10mg/kg, but within this dose range, there was no further increase in effectiveness with dose.
This confirms that passive long-term immunotherapy with the antibody NI-203.26C11 targeting toxic hIAPP oligomers specifically holds great therapeutic potential. Due to the lack of progression of the diabetic phenotype, the data concerning the short-term efficacy of antibody therapy with NI-203.26C11 could not be obtained in the course of this study. The LALA-PG rNI.26C11 treated Tg group had similar values as the Tg PBS and 0.3mg/kg treated groups concerning BW gain, FG as well as glucose and insulin AUC in the OGTTs. These values indicate a similar extent of β-cell function between these groups, implying that macrophage activation is a key mechanism of action for the clearance of cytotoxic hIAPP oligomers and the preservation of β-cell function. A novel method of target engagement was developed and provides us with tool of great potential for future research.

7. References

Abedini, Andisheh; Cao, Ping; Plesner, Annette; Zhang, Jinghua; He, Meilun; Derk, Julia et al. (2018): RAGE binds preamyloid IAPP intermediates and mediates pancreatic β cell proteotoxicity. In *The Journal of clinical investigation* 128 (2), pp. 682–698. DOI: 10.1172/JCI85210.

Abedini, Andisheh; Meng, Fanling; Raleigh, Daniel P. (2007): A single-point mutation converts the highly amyloidogenic human islet amyloid polypeptide into a potent fibrillization inhibitor. In *Journal of the American Chemical Society* 129 (37), pp. 11300–11301. DOI: 10.1021/ja072157y.

Abedini, Andisheh; Plesner, Annette; Cao, Ping; Ridgway, Zachary; Zhang, Jinghua; Tu, Ling-Hsien et al. (2016): Time-resolved studies define the nature of toxic IAPP intermediates, providing insight for anti-amyloidosis therapeutics. In *eLife* 5. DOI: 10.7554/eLife.12977.

Abedini, Andisheh; Schmidt, Ann Marie (2013): Mechanisms of islet amyloidosis toxicity in type 2 diabetes. In *FEBS letters* 587 (8), pp. 1119–1127. DOI: 10.1016/j.febslet.2013.01.017.

Adams, G. P.; Schier, R.; McCall, A. M.; Crawford, R. S.; Wolf, E. J.; Weiner, L. M.; Marks, J. D. (1998): Prolonged in vivo tumour retention of a human diabody targeting the extracellular domain of human HER2/neu. In *British journal of cancer* 77 (9), pp. 1405–1412.

Aitken, Jacqueline F.; Loomes, Kerry M.; Riba-Garcia, Isabel; Unwin, Richard D.; Prijic, Gordana; Phillips, Ashley S. et al. (2017): Rutin suppresses human-amylin/hIAPP misfolding and oligomer formation in-vitro, and ameliorates diabetes and its impacts in human-amylin/hIAPP transgenic mice. In *Biochemical and biophysical research communications* 482 (4), pp. 625–631. DOI: 10.1016/j.bbrc.2016.11.083.

Akter, Rehana; Cao, Ping; Noor, Harris; Ridgway, Zachary; Tu, Ling-Hsien; Wang, Hui et al. (2016): Islet Amyloid Polypeptide: Structure, Function, and Pathophysiology. In *Journal of Diabetes Research* 2016, p. 2798269. DOI: 10.1155/2016/2798269.

Anguiano, Magdalena; Nowak, Richard J.; Lansbury, Peter T. (2002): Protofibrillar islet amyloid polypeptide permeabilizes synthetic vesicles by a pore-like mechanism that may be relevant to type II diabetes. In *Biochemistry* 41 (38), pp. 11338–11343.

Arnelo, U.; Permert, J.; Adrian, T. E.; Larsson, J.; Westermark, P.; Reidelberger, R. D. (1996): Chronic infusion of islet amyloid polypeptide causes anorexia in rats. In *The American journal of physiology* 271 (6 Pt 2), R1654-9. DOI: 10.1152/ajpregu.1996.271.6.R1654.

Arrighi, H. Michael; Barakos, Jerome; Barkhof, Frederik; Tampieri, Donatella; Jack, Clifford; Melançon, Denis et al. (2016): Amyloid-related imaging abnormalities-haemosiderin (ARIA-H) in patients with Alzheimer's disease treated with bapineuzumab: a historical, prospective secondary analysis. In *Journal of neurology, neurosurgery, and psychiatry* 87 (1), pp. 106–112. DOI: 10.1136/jnnp-2014-309493.

Ashcroft, Frances M.; Rorsman, Patrik (2012): Diabetes mellitus and the β cell: the last ten years. In *Cell* 148 (6), pp. 1160–1171. DOI: 10.1016/j.cell.2012.02.010.

Bard, F.; Cannon, C.; Barbour, R.; Burke, R. L.; Games, D.; Grajeda, H. et al. (2000): Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. In *Nature medicine* 6 (8), pp. 916–919. DOI: 10.1038/78682.

Barth, Stephan W.; Riediger, Thomas; Lutz, Thomas A.; Rechkemmer, Gerhard (2003): Differential effects of amylin and salmon calcitonin on neuropeptide gene expression in the lateral hypothalamic area and the arcuate nucleus of the rat. In *Neuroscience letters* 341 (2), pp. 131–134.

Beeg, Marten; Stravalaci, Matteo; Romeo, Margherita; Carrá, Arianna Dorotea; Cagnotto, Alfredo; Rossi, Alessandro et al. (2016): Clusterin Binds to Aβ 1–42 Oligomers with High Affinity and Interferes with Peptide Aggregation by Inhibiting Primary and Secondary Nucleation. In *J. Biol. Chem.* 291 (13), pp. 6958–6966. DOI: 10.1074/jbc.M115.689539.

BELL, E. T. (1959): Hyalinization of the islets of Langerhans in nondiabetic individuals. In *The American journal of pathology* 35 (4), pp. 801–805.

Bellou, Vanesa; Belbasis, Lazaros; Tzoulaki, Ioanna; Evangelou, Evangelos (2018): Risk factors for type 2 diabetes mellitus: An exposure-wide umbrella review of meta-analyses. In *PLoS ONE* 13 (3). DOI: 10.1371/journal.pone.0194127.

Betsholtz, C.; Christmanson, L.; Engström, U.; Rorsman, F.; Jordan, K.; O'Brien, T. D. et al. (1990): Structure of cat islet amyloid polypeptide and identification of amino acid residues of potential significance for islet amyloid formation. In *Diabetes* 39 (1), pp. 118–122.

Betsholtz, C.; Christmansson, L.; Engström, U.; Rorsman, F.; Svensson, V.; Johnson, K. H.; Westermark, P. (1989): Sequence divergence in a specific region of islet amyloid polypeptide (IAPP) explains differences in islet amyloid formation between species. In *FEBS letters* 251 (1-2), pp. 261–264.

Boden, G.; Soeldner, J. S.; Gleason, R. E.; Marble, A. (1968): Elevated serum human growth hormone and decreased serum insulin in prediabetic males after intravenous tolbutamide and glucose. In *The Journal of clinical investigation* 47 (4), pp. 729–739. DOI: 10.1172/JCI105768.

Bolognesi, Benedetta; Kumita, Janet R.; Barros, Teresa P.; Esbjorner, Elin K.; Luheshi, Leila M.; Crowther, Damian C. et al. (2010): ANS binding reveals common features of cytotoxic amyloid species. In *ACS chemical biology* 5 (8), pp. 735–740. DOI: 10.1021/cb1001203.

Bongiovanni, Marie; Aprile, Francesco; Sormanni, Pietro; Vendruscolo, Michele (2018): A Rationally Designed Hsp70 Variant Rescues the Aggregation-Associated Toxicity of Human IAPP in Cultured Pancreatic Islet β-Cells. In *IJMS* 19 (5), p. 1443. DOI: 10.3390/ijms19051443.

Bonito-Oliva, Alessandra; Barbash, Shahar; Sakmar, Thomas P.; Graham, W. Vallen (2017): Nucleobindin 1 binds to multiple types of pre-fibrillar amyloid and inhibits fibrillization. In *Sci Rep* 7, p. 42880. DOI: 10.1038/srep42880.

Bram, Yaron; Frydman-Marom, Anat; Yanai, Inbal; Gilead, Sharon; Shaltiel-Karyo, Ronit; Amdursky, Nadav; Gazit, Ehud (2014): Apoptosis induced by islet amyloid polypeptide soluble oligomers is neutralized by diabetes-associated specific antibodies. In *Scientific reports* 4, p. 4267. DOI: 10.1038/srep04267.

References

Brender, Jeffrey R.; Salamekh, Samer; Ramamoorthy, Ayyalusamy (2012): Membrane disruption and early events in the aggregation of the diabetes related peptide IAPP from a molecular perspective. In *Accounts of chemical research* 45 (3), pp. 454–462. DOI: 10.1021/ar200189b.

Bucciantini, Monica; Giannoni, Elisa; Chiti, Fabrizio; Baroni, Fabiana; Formigli, Lucia; Zurdo, Jesús et al. (2002): Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. In *Nature* 416 (6880), pp. 507–511. DOI: 10.1038/416507a.

Burns, Alistair; Iliffe, Steve (2009): Dementia. In *BMJ (Clinical research ed.)* 338, b75. DOI: 10.1136/bmj.b75.

Butler, Alexandra E.; Jang, Jennifer; Gurlo, Tatyana; Carty, Maynard D.; Soeller, Walter C.; Butler, Peter C. (2004): Diabetes due to a progressive defect in beta-cell mass in rats transgenic for human islet amyloid polypeptide (HIP Rat): a new model for type 2 diabetes. In *Diabetes* 53 (6), pp. 1509–1516.

Butler, Alexandra E.; Janson, Juliette; Bonner-Weir, Susan; Ritzel, Robert; Rizza, Robert A.; Butler, Peter C. (2003): Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. In *Diabetes* 52 (1), pp. 102–110.

Caillon, Lucie; Hoffmann, Anais R. F.; Botz, Alexandra; Khemtemourian, Lucie (2016): Molecular Structure, Membrane Interactions, and Toxicity of the Islet Amyloid Polypeptide in Type 2 Diabetes Mellitus. In *Journal of Diabetes Research* 2016, p. 5639875. DOI: 10.1155/2016/5639875.

Cao, Ping; Abedini, Andisheh; Raleigh, Daniel P. (2013): Aggregation of islet amyloid polypeptide: from physical chemistry to cell biology. In *Current opinion in structural biology* 23 (1), pp. 82–89. DOI: 10.1016/j.sbi.2012.11.003.

Cao, Ping; Meng, Fanling; Abedini, Andisheh; Raleigh, Daniel P. (2010): The ability of rodent islet amyloid polypeptide to inhibit amyloid formation by human islet amyloid polypeptide has important implications for the mechanism of amyloid formation and the design of inhibitors. In *Biochemistry* 49 (5), pp. 872–881. DOI: 10.1021/bi901751b.

Carlson, Christopher; Estergard, Wahiba; Oh, Joonmi; Suhy, Joyce; Jack, Clifford R.; Siemers, Eric; Barakos, Jerome (2011): Prevalence of asymptomatic vasogenic edema in pretreatment Alzheimer's disease study cohorts from phase 3 trials of semagacestat and solanezumab. In *Alzheimer's & dementia : the journal of the Alzheimer's Association* 7 (4), pp. 396–401. DOI: 10.1016/j.jalz.2011.05.2353.

Carlson, Christopher; Siemers, Eric; Hake, Ann; Case, Michael; Hayduk, Roza; Suhy, Joyce et al. (2016): Amyloid-related imaging abnormalities from trials of solanezumab for Alzheimer's disease. In *Alzheimer's & dementia (Amsterdam, Netherlands)* 2, pp. 75–85. DOI: 10.1016/j.dadm.2016.02.004.

Casas, Sílvia; Gomis, Ramon; Gribble, Fiona M.; Altirriba, Jordi; Knuutila, Sakari; Novials, Anna (2007): Impairment of the ubiquitin-proteasome pathway is a downstream endoplasmic reticulum stress response induced by extracellular human islet amyloid polypeptide and contributes to pancreatic beta-cell apoptosis. In *Diabetes* 56 (9), pp. 2284–2294. DOI: 10.2337/db07-0178.

Chappel, M. S.; Isenman, D. E.; Everett, M.; Xu, Y. Y.; Dorrington, K. J.; Klein, M. H. (1991): Identification of the Fc gamma receptor class I binding site in human IgG through the use of

recombinant IgG1/IgG2 hybrid and point-mutated antibodies. In *Proceedings of the National Academy of Sciences of the United States of America* 88 (20), pp. 9036–9040.

Chen, Mei-Sha; Zhao, De-Sheng; Yu, Ye-Ping; Li, Wei-Wei; Chen, Yong-Xiang; Zhao, Yu-Fen; Li, Yan-Mei (2013): Characterizing the assembly behaviors of human amylin: a perspective derived from C-terminal variants. In *Chemical communications (Cambridge, England)* 49 (18), pp. 1799–1801. DOI: 10.1039/C2CC33432A.

Chimon, Sandra; Shaibat, Medhat A.; Jones, Christopher R.; Calero, Diana C.; Aizezi, Buzulagu; Ishii, Yoshitaka (2007): Evidence of fibril-like β-sheet structures in a neurotoxic amyloid intermediate of Alzheimer's β-amyloid. In *Nature structural & molecular biology* 14 (12), pp. 1157–1164. DOI: 10.1038/nsmb1345.

Chiti, Fabrizio; Dobson, Christopher M. (2006): Protein misfolding, functional amyloid, and human disease. In *Annual review of biochemistry* 75, pp. 333–366. DOI: 10.1146/annurev.biochem.75.101304.123901.

Chiti, Fabrizio; Dobson, Christopher M. (2017): Protein Misfolding, Amyloid Formation, and Human Disease: A Summary of Progress Over the Last Decade. In *Annual review of biochemistry* 86, pp. 27–68. DOI: 10.1146/annurev-biochem-061516-045115.

Clark, A.; Nilsson, M. R. (2004): Islet amyloid: a complication of islet dysfunction or an aetiological factor in Type 2 diabetes? In *Diabetologia* 47 (2), pp. 157–169. DOI: 10.1007/s00125-003-1304-4.

Cooper, G. J.; Leighton, B.; Dimitriadis, G. D.; Parry-Billings, M.; Kowalchuk, J. M.; Howland, K. et al. (1988): Amylin found in amyloid deposits in human type 2 diabetes mellitus may be a hormone that regulates glycogen metabolism in skeletal muscle. In *Proceedings of the National Academy of Sciences of the United States of America* 85 (20), pp. 7763–7766.

Couce, M.; Kane, L. A.; O'Brien, T. D.; Charlesworth, J.; Soeller, W.; McNeish, J. et al. (1996): Treatment with growth hormone and dexamethasone in mice transgenic for human islet amyloid polypeptide causes islet amyloidosis and beta-cell dysfunction. In *Diabetes* 45 (8), pp. 1094–1101.

Das, Pritam; Howard, Victor; Loosbrock, Nicole; Dickson, Dennis; Murphy, M. Paul; Golde, Todd E. (2003): Amyloid-beta immunization effectively reduces amyloid deposition in FcRgamma-/- knock-out mice. In *The Journal of neuroscience : the official journal of the Society for Neuroscience* 23 (24), pp. 8532–8538.

Daval, Marie; Bedrood, Sahar; Gurlo, Tatyana; Huang, Chang-jiang; Costes, Safia; Butler, Peter C.; Langen, Ralf (2010): The effect of curcumin on human islet amyloid polypeptide misfolding and toxicity. In *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis* 17 (3-4), pp. 118–128. DOI: 10.3109/13506129.2010.530008.

Deane, Rashid; Sagare, Abhay; Hamm, Katie; Parisi, Margaret; LaRue, Barbra; Guo, Huang et al. (2005): IgG-assisted age-dependent clearance of Alzheimer's amyloid beta peptide by the blood-brain barrier neonatal Fc receptor. In *The Journal of neuroscience : the official journal of the Society for Neuroscience* 25 (50), pp. 11495–11503. DOI: 10.1523/JNEUROSCI.3697-05.2005.

DeMattos, R. B.; Bales, K. R.; Cummins, D. J.; Dodart, J. C.; Paul, S. M.; Holtzman, D. M. (2001): Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases

brain A beta burden in a mouse model of Alzheimer's disease. In *Proceedings of the National Academy of Sciences of the United States of America* 98 (15), pp. 8850–8855. DOI: 10.1073/pnas.151261398.

Despa, Sanda; Margulies, Kenneth B.; Le Chen; Knowlton, Anne A.; Havel, Peter J.; Taegtmeyer, Heinrich et al. (2012): Hyperamylinemia contributes to cardiac dysfunction in obesity and diabetes: a study in humans and rats. In *Circulation research* 110 (4), pp. 598–608. DOI: 10.1161/CIRCRESAHA.111.258285.

Du, Weihong; Gong, Gehui; Wang, Wenji; Xu, Jufei (2017): Regulation of the aggregation behavior of human islet amyloid polypeptide fragment by titanocene complexes. In *J Biol Inorg Chem* 22 (7), pp. 1065–1074. DOI: 10.1007/s00775-017-1484-x.

Eguchi, K.; Manabe, I. (2013): Macrophages and islet inflammation in type 2 diabetes. In *Diabetes, obesity & metabolism* 15 Suppl 3, pp. 152–158. DOI: 10.1111/dom.12168.

Eguchi, Kosei; Nagai, Ryozo (2017): Islet inflammation in type 2 diabetes and physiology. In *The Journal of clinical investigation* 127 (1), pp. 14–23. DOI: 10.1172/JCI88877.

Ehrnhoefer, Dagmar E.; Bieschke, Jan; Boeddrich, Annett; Herbst, Martin; Masino, Laura; Lurz, Rudi et al. (2008): EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers. In *Nat Struct Mol Biol* 15 (6), pp. 558–566. DOI: 10.1038/nsmb.1437.

Ehses, Jan A.; Perren, Aurel; Eppler, Elisabeth; Ribaux, Pascale; Pospisilik, John A.; Maor-Cahn, Ranit et al. (2007): Increased number of islet-associated macrophages in type 2 diabetes. In *Diabetes* 56 (9), pp. 2356–2370. DOI: 10.2337/db06-1650.

Engel, Maarten F. M. (2009): Membrane permeabilization by Islet Amyloid Polypeptide. In *Chemistry and physics of lipids* 160 (1), pp. 1–10. DOI: 10.1016/j.chemphyslip.2009.03.008.

Fang, Xiaocui; Yousaf, Maryam; Huang, Qunxing; Yang, Yanlian; Wang, Chen (2018): Dual effect of PEG-PE micelle over the oligomerization and fibrillation of human islet amyloid polypeptide. In *Scientific reports* 8 (1), p. 4463. DOI: 10.1038/s41598-018-22820-w.

Fox, N.; Schrementi, J.; Nishi, M.; Ohagi, S.; Chan, S. J.; Heisserman, J. A. et al. (1993): Human islet amyloid polypeptide transgenic mice as a model of non-insulin-dependent diabetes mellitus (NIDDM). In *FEBS letters* 323 (1-2), pp. 40–44.

Franko, Andras; Rodriguez Camargo, Diana C.; Böddrich, Annett; Garg, Divita; Rodriguez Camargo, Andres; Rathkolb, Birgit et al. (2018): Epigallocatechin gallate (EGCG) reduces the intensity of pancreatic amyloid fibrils in human islet amyloid polypeptide (hIAPP) transgenic mice. In *Sci Rep* 8 (1), p. 429. DOI: 10.1038/s41598-017-18807-8.

Gao, Mimi; Estel, Kathrin; Seeliger, Janine; Friedrich, Ralf P.; Dogan, Susanne; Wanker, Erich E. et al. (2015): Modulation of human IAPP fibrillation: cosolutes, crowders and chaperones. In *Phys. Chem. Chem. Phys.* 17 (13), pp. 8338–8348. DOI: 10.1039/c4cp04682j.

Gao, Mimi; Winter, Roland (2015): The Effects of Lipid Membranes, Crowding and Osmolytes on the Aggregation, and Fibrillation Propensity of Human IAPP. In *Journal of Diabetes Research* 2015, p. 849017. DOI: 10.1155/2015/849017.

Geisler, John G.; Zawalich, Walter; Zawalich, Kathleen; Lakey, Jonathan R. T.; Stukenbrok, Hans; Milici, Anthony J.; Soeller, Walter C. (2002): Estrogen can prevent or reverse obesity and diabetes in mice expressing human islet amyloid polypeptide. In *Diabetes* 51 (7), pp. 2158–2169.

Glabe, Charles G. (2008): Structural classification of toxic amyloid oligomers. In *The Journal of biological chemistry* 283 (44), pp. 29639–29643. DOI: 10.1074/jbc.R800016200.

Goldsbury, C.; Goldie, K.; Pellaud, J.; Seelig, J.; Frey, P.; Müller, S. A. et al. (2000): Amyloid fibril formation from full-length and fragments of amylin. In *Journal of structural biology* 130 (2-3), pp. 352–362. DOI: 10.1006/jsbi.2000.4268.

Gorbenko, Galyna P.; Kinnunen, Paavo K. J. (2006): The role of lipid-protein interactions in amyloid-type protein fibril formation. In *Chemistry and physics of lipids* 141 (1-2), pp. 72–82. DOI: 10.1016/j.chemphyslip.2006.02.006.

Greenwald, Jason; Riek, Roland (2010): Biology of amyloid: structure, function, and regulation. In *Structure (London, England : 1993)* 18 (10), pp. 1244–1260. DOI: 10.1016/j.str.2010.08.009.

Guo, Jingjing; Li, Jiazhong; Zhang, Yan; Jin, Xiaojie; Liu, Huanxiang; Yao, Xiaojun (2013): Exploring the Influence of Carbon Nanoparticles on the Formation of β-Sheet-Rich Oligomers of IAPP22–28 Peptide by Molecular Dynamics Simulation. In *PLoS ONE* 8 (6), e65579. DOI: 10.1371/journal.pone.0065579.

Gurlo, T.; Rivera, J. F.; Butler, A. E.; Cory, M.; Hoang, J.; Costes, S.; Butler, Peter C. (2016): CHOP Contributes to, But Is Not the Only Mediator of, IAPP Induced β-Cell Apoptosis. In *Molecular endocrinology (Baltimore, Md.)* 30 (4), pp. 446–454. DOI: 10.1210/me.2015-1255.

Gurzov, Esteban N.; Wang, Bo; Pilkington, Emily H.; Chen, Pengyu; Kakinen, Aleksandr; Stanley, William J. et al. (2016): Inhibition of hIAPP Amyloid Aggregation and Pancreatic β-Cell Toxicity by OH-Terminated PAMAM Dendrimer. In *Small* 12 (12), pp. 1615–1626. DOI: 10.1002/smll.201502317.

Haataja, Leena; Gurlo, Tatyana; Huang, Chang J.; Butler, Peter C. (2008): Islet amyloid in type 2 diabetes, and the toxic oligomer hypothesis. In *Endocrine reviews* 29 (3), pp. 303–316. DOI: 10.1210/er.2007-0037.

Halban, Philippe A.; Polonsky, Kenneth S.; Bowden, Donald W.; Hawkins, Meredith A.; Ling, Charlotte; Mather, Kieren J. et al. (2014): β-cell failure in type 2 diabetes: postulated mechanisms and prospects for prevention and treatment. In *The Journal of clinical endocrinology and metabolism* 99 (6), pp. 1983–1992. DOI: 10.1210/jc.2014-1425.

Hardy, John; Selkoe, Dennis J. (2002): The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. In *Science (New York, N.Y.)* 297 (5580), pp. 353–356. DOI: 10.1126/science.1072994.

Hernández, Miriam García; Aguilar, Ana García; Burillo, Jesús; Oca, Raquel Gómez; Manca, Maria Antonietta; Novials, Ana et al. (2018): Pancreatic β cells overexpressing hIAPP impaired mitophagy and unbalanced mitochondrial dynamics. In *Cell death & disease* 9 (5), p. 481. DOI: 10.1038/s41419-018-0533-x.

Holliger, P.; Prospero, T.; Winter, G. (1993): "Diabodies": small bivalent and bispecific antibody fragments. In *Proceedings of the National Academy of Sciences of the United States of America* 90 (14), pp. 6444–6448.

Höppener, J. W.; Oosterwijk, C.; Nieuwenhuis, M. G.; Posthuma, G.; Thijssen, J. H.; Vroom, T. M. et al. (1999): Extensive islet amyloid formation is induced by development of Type II

diabetes mellitus and contributes to its progression: pathogenesis of diabetes in a mouse model. In *Diabetologia* 42 (4), pp. 427–434. DOI: 10.1007/s001250051175.

Höppener, Jo W. M.; Lips, Cees J. M. (2006): Role of islet amyloid in type 2 diabetes mellitus. In *The international journal of biochemistry & cell biology* 38 (5-6), pp. 726–736. DOI: 10.1016/j.biocel.2005.12.009.

Huang, Chang-jiang; Lin, Chia-Yu; Haataja, Leena; Gurlo, Tatyana; Butler, Alexandra E.; Rizza, Robert A.; Butler, Peter C. (2007): High expression rates of human islet amyloid polypeptide induce endoplasmic reticulum stress mediated beta-cell apoptosis, a characteristic of humans with type 2 but not type 1 diabetes. In *Diabetes* 56 (8), pp. 2016–2027. DOI: 10.2337/db07-0197.

Hugentobler, Leoni (2017): Human-derived antibody targeting pancreatic islet amyloid for the treatment of type 2 diabetes. University of Zurich. Institute of Veterinary Physiology. Available online at https://www.zora.uzh.ch/id/eprint/140872/1/Hugentobler_Leoni_Dissertation_2017.pdf.

Hull, Rebecca L.; Westermark, Gunilla T.; Westermark, Per; Kahn, Steven E. (2004): Islet amyloid: a critical entity in the pathogenesis of type 2 diabetes. In *The Journal of clinical endocrinology and metabolism* 89 (8), pp. 3629–3643. DOI: 10.1210/jc.2004-0405.

Idusogie, E. E.; Wong, P. Y.; Presta, L. G.; Gazzano-Santoro, H.; Totpal, K.; Ultsch, M.; Mulkerrin, M. G. (2001): Engineered antibodies with increased activity to recruit complement. In *Journal of immunology (Baltimore, Md. : 1950)* 166 (4), pp. 2571–2575.

International Diabetes Federation (2017): IDF Diabetes Atlas. 8th edition.

Jack, Clifford R.; Knopman, David S.; Jagust, William J.; Shaw, Leslie M.; Aisen, Paul S.; Weiner, Michael W. et al. (2010): Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. In *The Lancet. Neurology* 9 (1), pp. 119–128. DOI: 10.1016/S1474-4422(09)70299-6.

Jaikaran, E. T.; Clark, A. (2001): Islet amyloid and type 2 diabetes: from molecular misfolding to islet pathophysiology. In *Biochimica et biophysica acta* 1537 (3), pp. 179–203.

Janson, J.; Ashley, R. H.; Harrison, D.; McIntyre, S.; Butler, P. C. (1999): The mechanism of islet amyloid polypeptide toxicity is membrane disruption by intermediate-sized toxic amyloid particles. In *Diabetes* 48 (3), pp. 491–498.

Janson, J.; Soeller, W. C.; Roche, P. C.; Nelson, R. T.; Torchia, A. J.; Kreutter, D. K.; Butler, P. C. (1996): Spontaneous diabetes mellitus in transgenic mice expressing human islet amyloid polypeptide. In *Proceedings of the National Academy of Sciences of the United States of America* 93 (14), pp. 7283–7288.

Jayasinghe, Sajith A.; Langen, Ralf (2007): Membrane interaction of islet amyloid polypeptide. In *Biochimica et biophysica acta* 1768 (8), pp. 2002–2009. DOI: 10.1016/j.bbamem.2007.01.022.

Jeong, Keunhong; Chung, Woo Young; Kye, Young-Sik; Kim, Dongwook (2010): Cu(II) cyclen cleavage agent for human islet amyloid peptide. In *Bioorganic & medicinal chemistry* 18 (7), pp. 2598–2601. DOI: 10.1016/j.bmc.2010.02.045.

Jurgens, Catherine A.; Toukatly, Mirna N.; Fligner, Corinne L.; Udayasankar, Jayalakshmi; Subramanian, Shoba L.; Zraika, Sakeneh et al. (2011): β-cell loss and β-cell apoptosis in human type 2 diabetes are related to islet amyloid deposition. In *The American journal of pathology* 178 (6), pp. 2632–2640. DOI: 10.1016/j.ajpath.2011.02.036.

Kahn, S. E.; Andrikopoulos, S.; Verchere, C. B. (1999): Islet amyloid: a long-recognized but underappreciated pathological feature of type 2 diabetes. In *Diabetes* 48 (2), pp. 241–253.

Kamata, Kosuke; Mizukami, Hiroki; Inaba, Wataru; Tsuboi, Kentaro; Tateishi, Yoshinori; Yoshida, Taro; Yagihashi, Soroku (2014): Islet amyloid with macrophage migration correlates with augmented β-cell deficits in type 2 diabetic patients. In *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis* 21 (3), pp. 191–201. DOI: 10.3109/13506129.2014.937857.

Kaplon, Hélène; Reichert, Janice M. (2018): Antibodies to watch in 2018. In *mAbs* 10 (2), pp. 183–203. DOI: 10.1080/19420862.2018.1415671.

Kapurniotu, A. (2001): Amyloidogenicity and cytotoxicity of islet amyloid polypeptide. In *Biopolymers* 60 (6), pp. 438–459. DOI: 10.1002/1097-0282(2001)60:6<438::AID-BIP10182>3.0.CO;2-A.

Kayed, R.; Bernhagen, J.; Greenfield, N.; Sweimeh, K.; Brunner, H.; Voelter, W.; Kapurniotu, A. (1999): Conformational transitions of islet amyloid polypeptide (IAPP) in amyloid formation in vitro. In *Journal of molecular biology* 287 (4), pp. 781–796. DOI: 10.1006/jmbi.1999.2646.

Kim, Hai-Young; Cho, Min-Kyu; Kumar, Ashutosh; Maier, Elke; Siebenhaar, Carsten; Becker, Stefan et al. (2009): Structural properties of pore-forming oligomers of alpha-synuclein. In *Journal of the American Chemical Society* 131 (47), pp. 17482–17489. DOI: 10.1021/ja9077599.

Kim, Jinyoung; Cheon, Hwanju; Jeong, Yeon Taek; Quan, Wenying; Kim, Kook Hwan; Cho, Jae Min et al. (2014): Amyloidogenic peptide oligomer accumulation in autophagy-deficient β cells induces diabetes. In *The Journal of clinical investigation* 124 (8), pp. 3311–3324. DOI: 10.1172/JCI69625.

Koenigsknecht-Talboo, Jessica; Landreth, Gary E. (2005): Microglial phagocytosis induced by fibrillar beta-amyloid and IgGs are differentially regulated by proinflammatory cytokines. In *The Journal of neuroscience : the official journal of the Society for Neuroscience* 25 (36), pp. 8240–8249. DOI: 10.1523/JNEUROSCI.1808-05.2005.

Koning, E. J. de; Höppener, J. W.; Verbeek, J. S.; Oosterwijk, C.; van Hulst, K. L.; Baker, C. A. et al. (1994): Human islet amyloid polypeptide accumulates at similar sites in islets of transgenic mice and humans. In *Diabetes* 43 (5), pp. 640–644.

Krishnamurthy, Pavan K.; Rajamohamedsait, Hameetha B.; Gonzalez, Veronica; Rajamohamedsait, Wajitha J.; Ahmed, Nawal; Krishnaswamy, Senthilkumar; Sigurdsson, Einar M. (2016): Sex and Immunogen-Specific Benefits of Immunotherapy Targeting Islet Amyloid Polypeptide in Transgenic and Wild-Type Mice. In *Frontiers in endocrinology* 7, p. 62. DOI: 10.3389/fendo.2016.00062.

Laganowsky, Arthur; Liu, Cong; Sawaya, Michael R.; Whitelegge, Julian P.; Park, Jiyong; Zhao, Minglei et al. (2012): Atomic view of a toxic amyloid small oligomer. In *Science (New York, N.Y.)* 335 (6073), pp. 1228–1231. DOI: 10.1126/science.1213151.

Lee, Tae Yeon; Chei, Woo Suk; Ju, Heeyeon; Lee, Mi-Sook; Lee, Jung Weon; Suh, Junghun (2012): A Co(III) complex cleaving soluble oligomers of h-IAPP in the presence of polymeric aggregates of h-IAPP. In *Bioorganic & medicinal chemistry letters* 22 (17), pp. 5689–5693. DOI: 10.1016/j.bmcl.2012.06.089.

References

Lin, Chia-Yu; Gurlo, Tatyana; Kayed, Rakez; Butler, Alexandra E.; Haataja, Leena; Glabe, Charles G.; Butler, Peter C. (2007): Toxic human islet amyloid polypeptide (h-IAPP) oligomers are intracellular, and vaccination to induce anti-toxic oligomer antibodies does not prevent h-IAPP-induced beta-cell apoptosis in h-IAPP transgenic mice. In *Diabetes* 56 (5), pp. 1324–1332. DOI: 10.2337/db06-1579.

Lo, Megan; Kim, Hok Seon; Tong, Raymond K.; Bainbridge, Travis W.; Vernes, Jean-Michel; Zhang, Yin et al. (2017): Effector-attenuating Substitutions That Maintain Antibody Stability and Reduce Toxicity in Mice. In *J. Biol. Chem.* 292 (9), pp. 3900–3908. DOI: 10.1074/jbc.M116.767749.

Lorenzo, A.; Razzaboni, B.; Weir, G. C.; Yankner, B. A. (1994): Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus. In *Nature* 368 (6473), pp. 756–760. DOI: 10.1038/368756a0.

Ludwig, G.; Heitner, H. (1967): Zur Häufigkeit der Inselamyloidose des Pankreas beim Diabetes mellitus. In *Zeitschrift fur die gesamte innere Medizin und ihre Grenzgebiete* 22 (24), pp. 814–819.

Lutz, T. A.; Del Prete, E.; Scharrer, E. (1994): Reduction of food intake in rats by intraperitoneal injection of low doses of amylin. In *Physiology & behavior* 55 (5), pp. 891–895.

Lutz, Thomas A. (2006): Amylinergic control of food intake. In *Physiology & behavior* 89 (4), pp. 465–471. DOI: 10.1016/j.physbeh.2006.04.001.

M. Osto (2014): In vivo efficacy of human-derived anti-human islet amyloid polypeptide antibodies in transgenic rodent models of type 2 diabetes. Swiss Winter Conf. Ingestive Behav. St. Moritz, 2014.

M. Osto (2015): Human-derived antibody targeting pancreatic islet amyloid for the treatment of type 2 diabetes. Swiss Winter Conf. Ingestive Behav. St. Moritz, 2015.

Mannini, Benedetta; Mulvihill, Estefania; Sgromo, Caterina; Cascella, Roberta; Khodarahmi, Reza; Ramazzotti, Matteo et al. (2014): Toxicity of protein oligomers is rationalized by a function combining size and surface hydrophobicity. In *ACS chemical biology* 9 (10), pp. 2309–2317. DOI: 10.1021/cb500505m.

Martino, Luisa; Masini, Matilde; Bugliani, Marco; Marselli, Lorella; Suleiman, Mara; Boggi, Ugo et al. (2015): Mast cells infiltrate pancreatic islets in human type 1 diabetes. In *Diabetologia* 58 (11), pp. 2554–2562. DOI: 10.1007/s00125-015-3734-1.

Marzban, Lucy; Tomas, Alejandra; Becker, Thomas C.; Rosenberg, Lawrence; Oberholzer, Jose; Fraser, Paul E. et al. (2016): Erratum. Small Interfering RNA—Mediated Suppression of Proislet Amyloid Polypeptide Expression Inhibits Islet Amyloid Formation and Enhances Survival of Human Islets in Culture. Diabetes 2008;57:3045—3055. In *Diabetes* 65 (3), p. 818. DOI: 10.2337/db16-er03.

Matveyenko, A. V.; Butler, P. C. (2006): B-Cell Deficit Due to Increased Apoptosis in the Human Islet Amyloid Polypeptide Transgenic (HIP) Rat Recapitulates the Metabolic Defects Present in Type 2 Diabetes. In *Diabetes* 55 (7), pp. 2106–2114. DOI: 10.2337/db05-1672.

Meng, Fanling; Raleigh, Daniel P.; Abedini, Andisheh (2010): Combination of kinetically selected inhibitors in trans leads to highly effective inhibition of amyloid formation. In *Journal of the American Chemical Society* 132 (41), pp. 14340–14342. DOI: 10.1021/ja1046186.

Morgan, D. (2011): Immunotherapy for Alzheimer's disease. In *Journal of internal medicine* 269 (1), pp. 54–63. DOI: 10.1111/j.1365-2796.2010.02315.x.

Morgan, Dave (2006): Immunotherapy for Alzheimer's disease. In *Journal of Alzheimer's disease : JAD* 9 (3 Suppl), pp. 425–432.

Morgan, Dave (2009): The role of microglia in antibody-mediated clearance of amyloid-beta from the brain. In *CNS & neurological disorders drug targets* 8 (1), pp. 7–15.

Muff, R.; Bühlmann, N.; Fischer, J. A.; Born, W. (1999): An amylin receptor is revealed following co-transfection of a calcitonin receptor with receptor activity modifying proteins-1 or -3. In *Endocrinology* 140 (6), pp. 2924–2927. DOI: 10.1210/endo.140.6.6930.

Mukherjee, Abhisek; Morales-Scheihing, Diego; Butler, Peter C.; Soto, Claudio (2015): Type 2 diabetes as a protein misfolding disease. In *Trends in molecular medicine* 21 (7), pp. 439–449. DOI: 10.1016/j.molmed.2015.04.005.

Mukherjee, Abhisek; Morales-Scheihing, Diego; Salvadores, Natalia; Moreno-Gonzalez, Ines; Gonzalez, Cesar; Taylor-Presse, Kathleen et al. (2017): Induction of IAPP amyloid deposition and associated diabetic abnormalities by a prion-like mechanism. In *The Journal of experimental medicine* 214 (9), pp. 2591–2610. DOI: 10.1084/jem.20161134.

Ninomiya, Toshiharu (2014): Diabetes mellitus and dementia. In *Current diabetes reports* 14 (5), p. 487. DOI: 10.1007/s11892-014-0487-z.

Oskarsson, Marie E.; Paulsson, Johan F.; Schultz, Sebastian W.; Ingelsson, Martin; Westermark, Per; Westermark, Gunilla T. (2015): In vivo seeding and cross-seeding of localized amyloidosis: a molecular link between type 2 diabetes and Alzheimer disease. In *The American journal of pathology* 185 (3), pp. 834–846. DOI: 10.1016/j.ajpath.2014.11.016.

Padrick, S. B.; Miranker, A. D. (2001): Islet amyloid polypeptide: identification of long-range contacts and local order on the fibrillogenesis pathway. In *Journal of molecular biology* 308 (4), pp. 783–794. DOI: 10.1006/jmbi.2001.4608.

Pankiewicz, Joanna E.; Sadowski, Martin J. (2017): APOE genotype and Alzheimer's immunotherapy. In *Oncotarget* 8 (25), pp. 39941–39942. DOI: 10.18632/oncotarget.17990.

Park, Y. J.; Lee, S.; Kieffer, T. J.; Warnock, G. L.; Safikhan, N.; Speck, M. et al. (2012): Deletion of Fas protects islet beta cells from cytotoxic effects of human islet amyloid polypeptide. In *Diabetologia*. DOI: 10.1007/s00125-012-2451-2.

Pithadia, Amit S.; Bhunia, Anirban; Sribalan, Rajendran; Padmini, Vediappen; Fierke, Carol A.; Ramamoorthy, Ayyalusamy (2016): Influence of a curcumin derivative on hIAPP aggregation in the absence and presence of lipid membranes. In *Chemical communications (Cambridge, England)* 52 (5), pp. 942–945. DOI: 10.1039/c5cc07792c.

Piya, Milan K.; Tahrani, Abd A.; Barnett, Anthony H. (2010): Emerging treatment options for type 2 diabetes. In *British journal of clinical pharmacology* 70 (5), pp. 631–644. DOI: 10.1111/j.1365-2125.2010.03711.x.

References

Porat, Yair; Kolusheva, Sofiya; Jelinek, Raz; Gazit, Ehud (2003): The human islet amyloid polypeptide forms transient membrane-active prefibrillar assemblies. In *Biochemistry* 42 (37), pp. 10971–10977. DOI: 10.1021/bi034889i.

Prince, Martin; Ali, Gemma-Claire; Guerchet, Maëlenn; Prina, A. Matthew; Albanese, Emiliano; Wu, Yu-Tzu (2016): Recent global trends in the prevalence and incidence of dementia, and survival with dementia. In *Alzheimer's research & therapy* 8 (1), p. 23. DOI: 10.1186/s13195-016-0188-8.

Prince, Martin; Bryce, Renata; Albanese, Emiliano; Wimo, Anders; Ribeiro, Wagner; Ferri, Cleusa P. (2013): The global prevalence of dementia: a systematic review and metaanalysis. In *Alzheimer's & dementia : the journal of the Alzheimer's Association* 9 (1), 63-75.e2. DOI: 10.1016/j.jalz.2012.11.007.

Querfurth, Henry W.; LaFerla, Frank M. (2010): Alzheimer's disease. In *The New England journal of medicine* 362 (4), pp. 329–344. DOI: 10.1056/NEJMra0909142.

Reidelberger, R. D.; Arnelo, U.; Granqvist, L.; Permert, J. (2001): Comparative effects of amylin and cholecystokinin on food intake and gastric emptying in rats. In *American journal of physiology. Regulatory, integrative and comparative physiology* 280 (3), R605-11. DOI: 10.1152/ajpregu.2001.280.3.R605.

Richardson, S. J.; Willcox, A.; Bone, A. J.; Foulis, A. K.; Morgan, N. G. (2009): Islet-associated macrophages in type 2 diabetes. In *Diabetologia* 52 (8), pp. 1686–1688. DOI: 10.1007/s00125-009-1410-z.

Ritzel, Robert A.; Butler, Peter C. (2003): Replication increases beta-cell vulnerability to human islet amyloid polypeptide-induced apoptosis. In *Diabetes* 52 (7), pp. 1701–1708.

Ritzel, Robert A.; Meier, Juris J.; Lin, Chia-Yu; Veldhuis, Johannes D.; Butler, Peter C. (2007): Human islet amyloid polypeptide oligomers disrupt cell coupling, induce apoptosis, and impair insulin secretion in isolated human islets. In *Diabetes* 56 (1), pp. 65–71. DOI: 10.2337/db06-0734.

Rivera, J. F.; Gurlo, T.; Daval, M.; Huang, C. J.; Matveyenko, A. V.; Butler, P. C.; Costes, S. (2011): Human-IAPP disrupts the autophagy/lysosomal pathway in pancreatic β-cells: protective role of p62-positive cytoplasmic inclusions. In *Cell death and differentiation* 18 (3), pp. 415–426. DOI: 10.1038/cdd.2010.111.

Rojas, Joselyn; Bermudez, Valmore; Palmar, Jim; Martínez, María Sofía; Olivar, Luis Carlos; Nava, Manuel et al. (2018): Pancreatic Beta Cell Death: Novel Potential Mechanisms in Diabetes Therapy. In *Journal of Diabetes Research* 2018, p. 9601801. DOI: 10.1155/2018/9601801.

Schenk, D.; Barbour, R.; Dunn, W.; Gordon, G.; Grajeda, H.; Guido, T. et al. (1999): Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. In *Nature* 400 (6740), pp. 173–177. DOI: 10.1038/22124.

Schilling, Stephan; Rahfeld, Jens-Ulrich; Lues, Inge; Lemere, Cynthia A. (2018): Passive Aβ Immunotherapy: Current Achievements and Future Perspectives. In *Molecules (Basel, Switzerland)* 23 (5). DOI: 10.3390/molecules23051068.

Schlothauer, Tilman; Herter, Sylvia; Koller, Claudia Ferrara; Grau-Richards, Sandra; Steinhart, Virginie; Spick, Christian et al. (2016): Novel human IgG1 and IgG4 Fc-engineered antibodies

with completely abolished immune effector functions. In *Protein engineering, design & selection : PEDS* 29 (10), pp. 457–466. DOI: 10.1093/protein/gzw040.

Scrocchi, Louise A.; Chen, Yan; Waschuk, Stefko; Wang, Feng; Cheung, Sindy; Darabie, Audrey A. et al. (2002): Design of Peptide-based Inhibitors of Human Islet Amyloid Polypeptide Fibrillogenesis. In *Journal of molecular biology* 318 (3), pp. 697–706. DOI: 10.1016/S0022-2836(02)00164-X.

Seeliger, Janine; Werkmüller, Alexander; Winter, Roland (2013): Macromolecular crowding as a suppressor of human IAPP fibril formation and cytotoxicity. In *PLoS ONE* 8 (7), e69652. DOI: 10.1371/journal.pone.0069652.

Sevigny, Jeff; Chiao, Ping; Bussière, Thierry; Weinreb, Paul H.; Williams, Leslie; Maier, Marcel et al. (2016): The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. In *Nature* 537 (7618), pp. 50–56. DOI: 10.1038/nature19323.

Shigihara, Nayumi; Fukunaka, Ayako; Hara, Akemi; Komiya, Koji; Honda, Akira; Uchida, Toyoyoshi et al. (2014): Human IAPP-induced pancreatic β cell toxicity and its regulation by autophagy. In *The Journal of clinical investigation* 124 (8), pp. 3634–3644. DOI: 10.1172/JCI69866.

Simon, Gabriel M.; Niphakis, Micah J.; Cravatt, Benjamin F. (2013): Determining target engagement in living systems. In *Nature chemical biology* 9 (4), pp. 200–205. DOI: 10.1038/nchembio.1211.

Soeller, W. C.; Janson, J.; Hart, S. E.; Parker, J. C.; Carty, M. D.; Stevenson, R. W. et al. (1998): Islet amyloid-associated diabetes in obese A(vy)/a mice expressing human islet amyloid polypeptide. In *Diabetes* 47 (5), pp. 743–750.

Solomon, B.; Koppel, R.; Frankel, D.; Hanan-Aharon, E. (1997): Disaggregation of Alzheimer beta-amyloid by site-directed mAb. In *Proceedings of the National Academy of Sciences of the United States of America* 94 (8), pp. 4109–4112.

Solomon, B.; Koppel, R.; Hanan, E.; Katzav, T. (1996): Monoclonal antibodies inhibit in vitro fibrillar aggregation of the Alzheimer beta-amyloid peptide. In *Proceedings of the National Academy of Sciences of the United States of America* 93 (1), pp. 452–455.

Spanopoulou, Anna; Heidrich, Luzia; Chen, Hong-Ru; Frost, Christina; Hrle, Dean; Malideli, Eleni et al. (2018): Designed Macrocyclic Peptides as Nanomolar Amyloid Inhibitors Based on Minimal Recognition Elements. In *Angewandte Chemie (International ed. in English)*. DOI: 10.1002/anie.201802979.

Sparks, Samuel; Liu, Gai; Robbins, Kevin J.; Lazo, Noel D. (2012): Curcumin modulates the self-assembly of the islet amyloid polypeptide by disassembling α-helix. In *Biochemical and biophysical research communications* 422 (4), pp. 551–555. DOI: 10.1016/j.bbrc.2012.05.013.

Sperling, Reisa; Salloway, Stephen; Brooks, David J.; Tampieri, Donatella; Barakos, Jerome; Fox, Nick C. et al. (2012): Amyloid-related imaging abnormalities in patients with Alzheimer's disease treated with bapineuzumab: a retrospective analysis. In *The Lancet. Neurology* 11 (3), pp. 241–249. DOI: 10.1016/S1474-4422(12)70015-7.

Sperling, Reisa A.; Jack, Clifford R.; Black, Sandra E.; Frosch, Matthew P.; Greenberg, Steven M.; Hyman, Bradley T. et al. (2011): Amyloid-related imaging abnormalities in amyloid-

modifying therapeutic trials: recommendations from the Alzheimer's Association Research Roundtable Workgroup. In *Alzheimer's & dementia : the journal of the Alzheimer's Association* 7 (4), pp. 367–385. DOI: 10.1016/j.jalz.2011.05.2351.

Strohl, William R. (2018): Current progress in innovative engineered antibodies. In *Protein & cell* 9 (1), pp. 86–120. DOI: 10.1007/s13238-017-0457-8.

Stumvoll, Michael; Goldstein, Barry J.; van Haeften, Timon W. (2005): Type 2 diabetes: principles of pathogenesis and therapy. In *Lancet (London, England)* 365 (9467), pp. 1333–1346. DOI: 10.1016/S0140-6736(05)61032-X.

Sunde, M.; Blake, C. (1997): The structure of amyloid fibrils by electron microscopy and X-ray diffraction. In *Advances in protein chemistry* 50, pp. 123–159.

Tilakaratne, N.; Christopoulos, G.; Zumpe, E. T.; Foord, S. M.; Sexton, P. M. (2000): Amylin receptor phenotypes derived from human calcitonin receptor/RAMP coexpression exhibit pharmacological differences dependent on receptor isoform and host cell environment. In *The Journal of pharmacology and experimental therapeutics* 294 (1), pp. 61–72.

Tuomi, Tiinamaija; Santoro, Nicola; Caprio, Sonia; Cai, Mengyin; Weng, Jianping; Groop, Leif (2014): The many faces of diabetes: a disease with increasing heterogeneity. In *Lancet (London, England)* 383 (9922), pp. 1084–1094. DOI: 10.1016/S0140-6736(13)62219-9.

Uversky, Vladimir N. (2010): Targeting intrinsically disordered proteins in neurodegenerative and protein dysfunction diseases: another illustration of the D(2) concept. In *Expert review of proteomics* 7 (4), pp. 543–564. DOI: 10.1586/epr.10.36.

Uversky, Vladimir N.; Oldfield, Christopher J.; Dunker, A. Keith (2008): Intrinsically disordered proteins in human diseases: introducing the D2 concept. In *Annual review of biophysics* 37, pp. 215–246. DOI: 10.1146/annurev.biophys.37.032807.125924.

Uversky, Vladimir N.; Oldfield, Christopher J.; Midic, Uros; Xie, Hongbo; Xue, Bin; Vucetic, Slobodan et al. (2009): Unfoldomics of human diseases: linking protein intrinsic disorder with diseases. In *BMC genomics* 10 Suppl 1, S7. DOI: 10.1186/1471-2164-10-S1-S7.

van Haeften, T. W.; Dubbeldam, S.; Zonderland, M. L.; Erkelens, D. W. (1998): Insulin secretion in normal glucose-tolerant relatives of type 2 diabetic subjects. Assessments using hyperglycemic glucose clamps and oral glucose tolerance tests. In *Diabetes care* 21 (2), pp. 278–282.

Villemagne, Victor L.; Burnham, Samantha; Bourgeat, Pierrick; Brown, Belinda; Ellis, Kathryn A.; Salvado, Olivier et al. (2013): Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. In *The Lancet. Neurology* 12 (4), pp. 357–367. DOI: 10.1016/S1474-4422(13)70044-9.

Wang, Hui; Raleigh, Daniel P. (2014): General amyloid inhibitors? A critical examination of the inhibition of IAPP amyloid formation by inositol stereoisomers. In *PLoS ONE* 9 (9), e104023. DOI: 10.1371/journal.pone.0104023.

Wang, Li; Lei, Liyan; Li, Yang; Wang, Liping; Li, Fei (2014): A hIAPP-derived all-D-amino-acid inhibits hIAPP fibrillation efficiently at membrane surface by targeting α-helical oligomeric intermediates. In *FEBS letters* 588 (6), pp. 884–891. DOI: 10.1016/j.febslet.2014.02.020.

Wang, Xinhua; Mathieu, Mary; Brezski, Randall J. (2018): IgG Fc engineering to modulate antibody effector functions. In *Protein & cell* 9 (1), pp. 63–73. DOI: 10.1007/s13238-017-0473-8.

Webster, S. D.; Galvan, M. D.; Ferran, E.; Garzon-Rodriguez, W.; Glabe, C. G.; Tenner, A. J. (2001): Antibody-mediated phagocytosis of the amyloid beta-peptide in microglia is differentially modulated by C1q. In *Journal of immunology (Baltimore, Md. : 1950)* 166 (12), pp. 7496–7503.

Westermark, P. (1972): Quantitative studies on amyloid in the islets of Langerhans. In *Upsala journal of medical sciences* 77 (2), pp. 91–94.

Westermark, P.; Engström, U.; Johnson, K. H.; Westermark, G. T.; Betsholtz, C. (1990): Islet amyloid polypeptide: pinpointing amino acid residues linked to amyloid fibril formation. In *Proceedings of the National Academy of Sciences of the United States of America* 87 (13), pp. 5036–5040.

Westermark, Per; Andersson, Arne; Westermark, Gunilla T. (2011): Islet amyloid polypeptide, islet amyloid, and diabetes mellitus. In *Physiological reviews* 91 (3), pp. 795–826. DOI: 10.1152/physrev.00042.2009.

Westermark, Per; Benson, Merrill D.; Buxbaum, Joel N.; Cohen, Alan S.; Frangione, Blas; Ikeda, Shu-Ichi et al. (2007): A primer of amyloid nomenclature. In *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis* 14 (3), pp. 179–183. DOI: 10.1080/13506120701460923.

Wijesekara, N.; Ahrens, R.; Wu, L.; Ha, K.; Liu, Y.; Wheeler, M. B.; Fraser, P. E. (2015): Islet amyloid inhibitors improve glucose homeostasis in a transgenic mouse model of type 2 diabetes. In *Diabetes Obes Metab* 17 (10), pp. 1003–1006. DOI: 10.1111/dom.12529.

Xu, Jufei; Gong, Gehui; Huang, Xiangyi; Du, Weihong (2018): Schiff base oxovanadium complexes resist the assembly behavior of human islet amyloid polypeptide. In *Journal of inorganic biochemistry* 186, pp. 60–69. DOI: 10.1016/j.jinorgbio.2018.05.006.

Xu, Zhi-Xue; Zhang, Qiang; Ma, Gong-Li; Chen, Cong-Heng; He, Yan-Ming; Xu, Li-Hui et al. (2016): Influence of Aluminium and EGCG on Fibrillation and Aggregation of Human Islet Amyloid Polypeptide. In *Journal of Diabetes Research* 2016 (1), pp. 1–14. DOI: 10.1155/2016/1867059.

Yagui, K.; Yamaguchi, T.; Kanatsuka, A.; Shimada, F.; Huang, C. I.; Tokuyama, Y. et al. (1995): Formation of islet amyloid fibrils in beta-secretory granules of transgenic mice expressing human islet amyloid polypeptide/amylin. In *European journal of endocrinology* 132 (4), pp. 487–496.

Yanagi, Kotaro; Ashizaki, Mizue; Yagi, Hisashi; Sakurai, Kazumasa; Lee, Young-Ho; Goto, Yuji (2011): Hexafluoroisopropanol induces amyloid fibrils of islet amyloid polypeptide by enhancing both hydrophobic and electrostatic interactions. In *J. Biol. Chem.* 286 (27), pp. 23959–23966. DOI: 10.1074/jbc.M111.226688.

Yang, Y.; Song, W. (2013): Molecular links between Alzheimer's disease and diabetes mellitus. In *Neuroscience* 250, pp. 140–150. DOI: 10.1016/j.neuroscience.2013.07.009.

Young, Lydia M.; Cao, Ping; Raleigh, Daniel P.; Ashcroft, Alison E.; Radford, Sheena E. (2014): Ion mobility spectrometry-mass spectrometry defines the oligomeric intermediates in amylin

amyloid formation and the mode of action of inhibitors. In *Journal of the American Chemical Society* 136 (2), pp. 660–670. DOI: 10.1021/ja406831n.

Yousaf, Maryam; Huang, Huan; Li, Ping; Wang, Chen; Yang, Yanlian (2017): Fluorine Functionalized Graphene Quantum Dots as Inhibitor against hIAPP Amyloid Aggregation. In *ACS Chem. Neurosci.* 8 (6), pp. 1368–1377. DOI: 10.1021/acschemneuro.7b00015.

Zhang, Shaoping; Liu, Junxi; Dragunow, Michael; Cooper, Garth J. S. (2003): Fibrillogenic amylin evokes islet beta-cell apoptosis through linked activation of a caspase cascade and JNK1. In *J. Biol. Chem.* 278 (52), pp. 52810–52819. DOI: 10.1074/jbc.M308244200.

Zhang, Yue; Yu, Xiao-Lin; Zhu, Jie; Liu, Shu-Ying; Liu, Xiang-Meng; Dong, Quan-Xiu et al. (2018): Intravenous immunoglobulin improves glucose control and β-cell function in human IAPP transgenic mice by attenuating islet inflammation and reducing IAPP oligomers. In *International immunopharmacology* 54, pp. 145–152. DOI: 10.1016/j.intimp.2017.11.012.

Zierath, J. R.; Galuska, D.; Engström, A.; Johnson, K. H.; Betsholtz, C.; Westermark, P.; Wallberg-Henriksson, H. (1992): Human islet amyloid polypeptide at pharmacological levels inhibits insulin and phorbol ester-stimulated glucose transport in in vitro incubated human muscle strips. In *Diabetologia* 35 (1), pp. 26–31.

Zou, Yu; Qian, Zhenyu; Sun, Yunxiang; Wei, Guanghong; Zhang, Qingwen (2017): Orcein-Related Small Molecule O4 Destabilizes hIAPP Protofibrils by Interacting Mostly with the Amyloidogenic Core Region. In *The journal of physical chemistry. B* 121 (39), pp. 9203–9212. DOI: 10.1021/acs.jpcb.7b08652.

Zraika, S.; Hull, R. L.; Udayasankar, J.; Aston-Mourney, K.; Subramanian, S. L.; Kisilevsky, R. et al. (2009): Oxidative stress is induced by islet amyloid formation and time-dependently mediates amyloid-induced beta cell apoptosis. In *Diabetologia* 52 (4), pp. 626–635. DOI: 10.1007/s00125-008-1255-x.

8. Figures

Figures

9. Tables

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